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CONTENTS

The Cuprammonium washes, their preparation, biological properties, and application ..... O. Butler 235
Bacillus morulans N. Sp. A bacterial organism found associated with curly top of the sugar beet ..... P. A. Boncquet 269
A new apparatus for aseptic ultrafiltration ..... Ralph E. Smith 290
Factors affecting the parasitism of Ustilago Zeæ ..... F. J. Pieмeisel 294
Sclerotium bataticola. The cause of a fruit-rot of peppers ..... William H. Martin 309
A Nectria parasitic on Norway maple ..... Mrs. T. Cook 314
Phytopathological Notes 316
Literature on plant diseases 332

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PHYTOPATHOLOGY

VOLUME VII NUMBER 4

AUGUST, 1917

THE CUPRAMMONIUM WASHES

Their Preparation, Biological Properties, and Application

O. Butler

With Plates III to X

A number of cuprammoniums have been recommended as fungicides, but, despite the fact that they form, or it would be more accurate to say, could be made to form, clear solutions and leave, on drying, inconspicuous spots on the foliage, no single one has sufficiently met the desiderata of practice to become generally employed. Practice demands that a fungicide be non-toxic to the sprayed plant at the strength at which it is most efficient and effective, but the cuprammoniums have the reputation of being unduly toxic and of not being as effective though more efficient than Bordeaux mixtures.

The toxicity of the cuprammoniums has been ascribed to soluble copper (Millardet[1] being notably a proponent of this view), to excess ammonia, and to ammonium sulphate. The prevailing opinion regarding the ineffectiveness of the cuprammoniums is based on their behavior relatively to Bordeaux mixtures which, in fact, is comparing solutions weak in copper with mixtures considerably stronger. Hawkins,[2] for instance, compared a cuprammonium containing 0.053 per cent copper with Bordeaux mixture containing 0.18 to 0.25 per cent copper. The question may, therefore, be asked, are any or is any one of the cuprammoniums worthy of being retained amongst the fungicides? In order to satisfactorily answer this question it will be necessary to study: (1) The composition and preparation of the different washes. (2) The relative toxicity of the different washes, and the conditions affecting the same.

[1] Millardet, A., and Gayon, U. Les divers procédés de traitement du mildiou par les composés cuivreux. Journ. Agr. prat. **1**: 729. 1887.

[2] Hawkins, L. A. Grape-spraying experiments in Michigan in 1909. U. S. Dept. Agr. Circ. 65. 1909.

(3) The relative efficiency and effectiveness of the cuprammoniums. This we will now proceed to do.

I. CHEMISTRY AND METHODS OF PREPARATION OF THE CUPRAMMONIUMS

In the preparation of the cuprammonium washes met with in practice either ammonium hydroxid or ammonium carbonate are employed as the solvent, the solute being, in the case of the former solvent, either copper turnings, i.e., metallic copper, copper sulphate, the basic copper carbonate malachite ($CuCO_3$. $Cu(OH)_2$), or the basic copper carbonate of Burgundy mixture (2 $CuCO_3$. 3 $Cu(OH)_2$)[3] and in the case of the latter the copper salts mentioned, together with basic copper sulphate and cuprammonium sulphate ($CuSO_4$. 4 NH_3. H_2O). With the exception of the copper sulphate-ammonia wash which is a cuprammonium sulphate, all the other washes prepared with ammonium hydroxid produce cuprammoniums of very similar, if not identic composition and form a well characterized group; similarly the washes prepared with ammonium carbonate are also compositionally so nearly alike as to form a well defined group. The former are cuprammonium hydrates, the latter cuprammonium carbonates. Since cuprammonium sulphate was the first introduced of the cuprammonium washes and the cuprammonium hydrates were introduced prior to the cuprammonium carbonates, it will be possible both to retain a group distribution and consider the several fungicides in chronological order.

A. Cuprammonium sulphate washes

Copper sulphate and ammonia. The copper sulphate and ammonia wash (eau céleste) was the first introduced of the cuprammonium fungicides and still remains, taking the world at large, the best known. It is the most easily prepared and at the same time the most unstable, even when sufficient ammonium hydroxid is employed to give a clear solution. The fungicide was introduced by Audoynaud[4] in 1885, but the original formula which is as follows:

	part
Cupric sulphate	1
Ammonium hydroxid sp. gr. 90	0.769[5] by volume
Water to	100

[3] Bedford, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. **11**: 86. 1910.

[4] Audoynaud, A. Le mildiou et les composés cupriques. Progrès agr. et vit. 1885.

[5] Equivalent of ammonium hydrate sp. gr. 92, 1 part by volume called for in the original formula.

proved injurious to vegetation and has suffered more or less marked modifications at the hands of subsequent writers as will be seen from a perusal of table 1.

TABLE 1

Strength of copper sulphate and ammonia wash and ratio cupric sulphate: ammonium hydroxid recommended by different authors

STRENGTH IN CUPRIC SULPHATE	RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM HYDROXID SP. GR. 0.90}}$
per cent	
0.26	1:1.50
0.50	1:0.76
0.50	1:1.06
0.50	1:1.70
0.50	1:1.14
1.00	1:0.73
1.00	1:1.06
1.00	1:1.46

The formulae given in table 1 do not necessarily represent the composition of the fungicide when put in service, however, for we find authors recommending that it be allowed to stand after being made for a few hours, half a day or even several days[6] so that the excess of ammonium hydroxid may pass off. Nor do the formulae permit, except in one instance, i.e., in the case of the wash containing 1 per cent cupric sulphate and a ratio cupric sulphate-ammonium hydroxid of 1:1.46, of the fungicide being applied as a perfectly clear solution which is essential if the copper is to be deposited on the sprayed foliage in the proper physical and chemical states. Millardet[7] long ago pointed out that Audoynaud's formula was in this respect defective and increased the amount of ammonium hydroxid so as to give a ratio of 1:1.53 instead of 1:0.769. The ratio cupric sulphate ammonium hydroxid required to give a clear 1 per cent solution (and Millardet's figures are correct) has to be greatly increased in order to prevent a precipitate forming when washes containing less than 1 per cent cupric sulphate are employed. The Duke of Bedford and Pickering[8] have shown that the amount of ammonium hydroxid required increases very considerably with the dilution and the data presented in table 2 confirms this view. But while the data given in the table show a very considerable increase in the amount of ammonium

[6] Viala, P. Les maladies de la vigne, ed. 3, p. 143. 1893.

[7] Millardet, A., and David, E. Essais comparatifs de divers procédés de traitement du mildiou. Compte-rendu Congrès nationale viticole, Bordeaux. Appendix, 60. 1886.

[8] Woburn Exp. Fruit Farm Rept. **11**: 18. 1910.

TABLE 2

Amount of ammonium hydroxid required to give clear solutions of copper sulphate and ammonia of different strengths in cupric sulphate together with the corresponding ratios cupric sulphate: ammonium hydroxide and percentages of ammonia

STRENGTH IN CUPRIC SULPHATE	AMOUNT AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION[1]	RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM HYDROXID}}$	STRENGTH OF SOLUTION IN NH_3 INDICATIVE ONLY
per cent	*per cent*		*per cent*
1.0	1.5	1:1.5	0.386
0.5	1.0	1:2.0	0.255
0.2	0.8	1:4.0	0.204
0.1	0.7	1:7.0	0.179

[1]The data given under the heading amount of ammonium or ammonium carbonate, as the case may be, required to give a clear solution indicates the amount of the salt required to give a clear solution for a period of time of not less than two hours in a closed vessel.

hydroxid relative to the copper, it also brings out equally clearly that the absolute amount of ammonia[9] in solution decreases as the concentration of the copper is lowered. It may also be well to add that owing to the extreme volatility of ammonium hydroxid the figures given in the table will have to be increased at temperatures much above 20°C. and conversely may be somewhat decreased for temperatures below 20°C.

The copper sulphate and ammonia wash may be most conveniently prepared by adding the ammonium hydroxid required to a strong solution of copper sulphate and diluting immediately after the precipitate first formed has dissolved, though the wash may be satisfactorily prepared even when the relative dilution of the salts varies within wide limits. It is generally considered that the copper and ammonia wash (and the other cuprammoniums) are best prepared with soft water since the solutions are decomposed by hard water. The amount of copper precipitated when hard water is used is, however, entirely negligible in practice as may be judged from table 3.[10]

TABLE 3

Amount of copper precipitated from the copper sulphate and ammonia wash by waters of different degrees of hardness

SOURCE OF WATER	HARDNESS OF WATER HYDROTIMETRIC DEGREES	COPPER PRECIPITATED
		per cent
City of Bordeaux	26.0	0.00086
Gayon well	92.0	0.00406

[9]The term ammonia is used to denote NH_3 unless the context indicates the connotation NH_4OH.

[10]Millardet, A., and Gayon, U. Les divers procédés de traitement du mildiou par les composés cuivreux. Journ. Agr. prat. **1**: 732. 1887.

When ammonium hydroxid is slowly added to a strong solution of cupric sulphate a precipitate of basic cupric sulphate ($CuSO_4$. 2 $Cu(OH)_2$) is thrown down which dissolves in an excess of the reagent forming a deep blue solution, the copper being then in the form $CuSO_4$. 3 NH_3. 2 H_2O[11] or, according to the more commonly accepted view $CuSO_4$. 4 NH_3. H_2O a salt which decomposes readily on volatilization of ammonia or on dilution, the copper being precipitated as a basic sulphate. When the fungicide dries upon foliage the copper is deposited as a basic sulphate mixed with a little ammonium sulphate in the ratio of 1:0.35; in other words only a very small amount of the latter salt can be present even when a 1 per cent solution is sprayed on foliage as will be clearly seen from a study of the reactions involved which are as follows:[12]

(1) $3\ CuSO_4\ 5\ H_2O + 4\ NH_4\ OH = CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)_2SO_4 + 15\ H_2O.$

(2) $CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)_2SO_4 + \frac{8\ NH_4OH}{\text{in excess}} = 3\ (CuSO_4.\ 4\ NH_3.H_2O) + 9\ H_2O.$

(3) $3\ (CuSO_4.\ 4\ NH_3.H_2O) + H_2O = CuSO_4.\ 2\ Cu(OH)_2 + 2\ (NH_4)SO_4 + 8\ NH_3$

B. Cuprammonium hydrate washes

A cuprammonium hydrate is formed when metallic copper, cupric oxide, cupric hydrate, malachite, or the basic cupric carbonate of Burgundy mixture are dissolved in ammonium hydroxid, and washes have been used in practice prepared from copper and all the copper salts mentioned with the exceptions of cupric oxid and hydrate. All the cuprammonium hydroxid washes decompose on dilution or on volatilization of ammonia with formation of cupric hydrate, the copper also being deposited in this form when the fungicides dry spontaneously on foliage. The cuprammonium hydrate washes are more stable than cuprammonium sulphate.

Copper and ammonia wash. The copper and ammonia wash, or Schweizer's reagent, was first introduced as a fungicide by Bellot des Minières in 1887[13] but despite the fact that the results he obtained are said to have been highly satisfactory it is practically unknown in the literature.

For the preparation of the copper and ammonia wash a very large amount of ammonium hydroxid is required and the copper must be acted

[11] Bedford, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. **11**: 17. 1910.

[12] Chester, F. D. The copper fungicides. Journ. Myc. **6**: 23. 1891.

[13] Bellot des Miniéres, H. Ammoniure de cuivre et parasites de la vigne. 1887.

on in the presence of air or traces of ammonium salts. Bellot des Minières employed the former method and prepared a stock solution which was diluted at time of use so as to contain 0.25 to 0.75 per cent metallic copper, i.e., the copper equivalent of a 1 to 3 per cent Bordeaux mixture. The stock solution was made as follows:

	parts
Copper turnings	1
Ammonia, sp. gr. 0.9	119 by volume

A stock solution prepared in the manner indicated can be diluted with water to 0.0317 per cent copper without a precipitate forming within a period of two hours. At this dilution, however, the solution contains 0.97 per cent ammonia which, as a glance at table 4 will show, is much higher than in the other cuprammoniums of equivalent strength in copper. The fact coupled with the difficulties incident to the preparation of the stock solution has seriously militated against the employment of the fungicide in practice, the advantages incident to its use, i.e., an adhesiveness equal to the copper sulphate and ammonia wash[14] and lesser injuriousness to the grape,[15] being not sufficiently compensatory to outweigh the drawbacks.

TABLE 4

Amount of ammonia required to give clear solutions of the cuprammonium washes when containing 0.0317 per cent metallic copper

NAME OF FUNGICIDE	AMOUNT NH_3 REQUIRED TO GIVE A CLEAR SOLUTION
	per cent
Copper-ammonia	0.9772
Copper sulphate-ammonia	0.179
Copper sulphate-ammonium carbonate	0.049
Malachite-ammonia	0.6821
Burgundy mixture-ammonia	0.360
Malachite-ammonium carbonate	0.047
Burgundy mixture-ammonium carbonate	0.038

Burgundy mixture and ammonia. This the earliest copper carbonate and ammonia wash employed in practice was introduced by Patrigeon in 1887[16] and consists simply in dissolving the basic copper carbonate of Burgundy mixture directly in the mother liquor by means of ammonium hydroxid. The wash is used to some extent in practice and

[14] Foëx, G. Cours complet de Viticulture ed. 4. 577.

[15] Foëx, G. The same, p. 578.

[16] Patrigeon, G. Nouveaux procédés de traitement du mildiou. Journ. agr. prat. **1**: 882. 1887.

is known in the United States under the name of modified eau céleste. The formula originally proposed was as follows:

	parts
Copper sulphate	1
Sodium carbonate	1.5
Ammonia sp. gr. 0.9	0.769 by volume
Water to	100

Patrigeon's formula has not suffered any marked modifications at the hands of the various authors who mention it as will be seen from table 5.

TABLE 5

Strength of Burgundy mixture-ammonia and corresponding ratios copper sulphate sodium carbonate, and copper sulphate ammonium hydroxid recommended by different authors

STRENGTH IN CUPRIC SULPHATE	RATIO $\frac{\text{COPPER SULPHATE}}{\text{SODIUM CARBONATE}}$	RATIO $\frac{\text{COPPER SULPHATE}}{\text{AMMONIUM HYDROXID}}$
per cent		
0.25	1:1.20	1:0.83
0.30	1:1.25	1:0.83
0.50	1:1.25	1:0.83
0.50	1:1.25	1:1.04
1.0	1:1	1:0.73
1.1	1:1.25	1:0.78

The amount of ammonia called for in the various formulae for the preparation of modified eau céleste is never sufficient to give clear solutions. The basic carbonate of copper of Burgundy mixture, separated from the mother liquor by decantation is, however, readily soluble in ammonium hydroxid, as may be gathered from table 6.

TABLE 6

Amount of ammonium hydroxid required to give clear solutions of modified eau céleste

STRENGTH IN COPPER SULPHATE	AMOUNT OF AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*	*per cent*
1.00	4	1.029
0.50	3	0.772
0.25	2.5	0.642
0.10	1.4	0.360

Burgundy mixture dissolves in ammonium hydroxid, forming cuprammonium hydrate and is not a mixture as Chester[17] believed of cupram-

[17] Chester, F. D. Report of the Mycologist. Delaware Agr. Exp. Sta. Rept. **4**: 68. 1891.

monium hydrate and carbonate. Modified eau céleste does not give rise to malachite on decomposing thus indicating absence of cuprammonium carbonate. When the wash dries upon sprayed foliage the copper is deposited as an hydrate, mixed with some sodium sulphate and bicarbonate unless the copper carbonate is separated from the mother liquor and levigated before being dissolved as is sometimes recommended.

Copper carbonate and ammonia. The copper carbonate and ammonia wash, or ammoniacal copper carbonate was introduced by Gastine[18] and in American fungicide literature is the cuprammonium most commonly met with.

Malachite dissolves in ammonium hydroxid forming a solution which, according to the Duke of Bedford and Pickering,[19] consists mainly of cuprammonium carbonate, while Chester[20] is of the opinion that both cuprammonium carbonate and hydrate are formed. As, however, the malachite ammonia wash does not deposit malachite on standing, I incline to the view that the copper is present solely as cuprammonium hydrate.

Malachite dissolves sparingly in ammonium hydroxid, the total amount entering into solution being less in a strong than a weak concentration of ammonia. Penny[21] for instance, found that a 42.68 per cent ammonium hydroxid dissolved, per gram weight of ammonia, 0.01329 grams of metallic copper while under similar conditions a 21.38 per cent solution dissolved 0.3132 grams and a 3.20 per cent solution 1.063 grams metallic copper respectively. These results, unfortunately for practice, can not be obtained by dissolving a given quantity of malachite in the suitable corresponding strength of ammonium hydroxid. In order to obtain the maximum solvent action it is necessary to use a very large excess of malachite, "even five fold or more" which introduces obvious difficulties that can not be turned except in a very empirical and unsatisfactory manner.

The malachite-ammonia wash was, as I have already indicated, proposed by Gastine whose formula was as follows:

	parts
Malachite	0.06
Ammonia sp. gr. 0.90	0.769
Water to	100

The above formula has been more or less modified at the hands of subsequent writers, as will be seen from table 7.

[18] Gastine, G. Emploi du carbonate ammoniacal de cuivre contre le peronospora. Prog. agr. et vit. **8**: 1887.

[19] Woburn Exp. Fruit Farm Rept. **11**: 21. 1910.

[20] The same, p. 68.

[21] Penny, C. L. The preparation of ammoniacal solution of copper carbonate. Delaware Agr. Exp. Sta. Bul. **22**: 5. 1893.

TABLE 7

Strength of malachite-ammonia wash and corresponding ratios malachite: ammonium hydroxid recommended by different authors

STRENGTH IN MALACHITE	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM HYDROXID}}$
per cent	
0.045	1:22.2
0.046	1:5.5
0.060	1:12.8
0.075	1:11.3
0.078	1:10
0.078	1:13.2
0.086	1:10
0.093	1:8.7
0.093	1:5.5
0.097	1:10
0.097	1:13.2
0.100	1:1.23
0.100	1:11
0.125	1:8
0.200	1:8

Neither in the original formula nor any of the subsequent ones that have come to my knowledge is the ratio malachite-ammonium hydroxid such as to insure complete dissolution of the copper salt. In order to dissolve the malachite completely, at least within a reasonable time, the ratio must be increased to 1:30. It is therefore clear that, as usually prepared, the wash either contains less copper than the formulae call for, or if the undissolved malachite is incorporated in the wash then the copper will be placed on the plants partly in the form of malachite and partly as a copper hydrate. In order to obviate these difficulties I have employed a stock solution prepared as follows:

	parts
Malachite	1
Ammonia sp. gr. 90	30 by volume
Water	20 by volume

In preparing the stock solution water must be used as otherwise the solution would prove unstable, decomposing with formation of cupric oxid. A stock solution containing as little as five parts water may be prepared and probably more water than the formula calls for could be used, though it will be perfectly obvious that there is no object in making a stock solution unduly dilute. In fact, *ceteris paribus* highly concentrated solutions are to be preferred.

The stock solution as above prepared is quite stable and may be diluted very considerably without decomposing, but contains more ammonia than

TABLE 8

Amount of ammonium hydroxid required to give clear solutions of malachite-ammonium hydroxid

STRENGTH IN TERMS OF COPPER SULPHATE	AMOUNT AMMONIUM HYDROXID REQUIRED TO GIVE CLEAR SOLUTION	STRENGTH OF SOLUTION IN NH_3
per cent	*cc.*	*per cent*
1	26.5	6.82
0.5	13.25	3.41
0.25	6.62	1.70
0.10	2.65	0.682

modified eau céleste for equal percentages of copper (Cu) as may be seen by comparing table 8 with table 6. There is, therefore, no justification, since sodium sulphate and bicarbonate are not injurious at the concentrations at which the wash may be employed in practice, in the preference accorded the malachite-ammonia wash, as the copper occurs in the same form in both. The difference in favor of modified eau céleste is not only marked, however, in the formulae I have used, but is also favorable to the latter when we compare the formulae of authors. Taking the extremes met with we find the results shown in table 9 which are even more favorable to modified eau céleste than in the case of my formulae. And when we consider the cost of the unit copper employed (exclusive of labor) in modified eau céleste and malachite-ammonia we obtain a ratio of 1:3 which is so significant as to require no comment.

TABLE 9

Extreme percentages of ammonia met with in the formulae of authors for the preparation of modified eau céleste (A) and malachite-ammonia (B) respectively

STRENGTH OF SOLUTION IN TERMS OF COPPER SULPHATE	STRENGTH OF SOLUTION IN NH_3	
	A	B
per cent	*per cent*	*per cent*
0.25	0.051	0.564
	0.073	0.141
1.1	0.206	0.707
	0.294	2.828

C. Cuprammonium carbonate washes

Cuprammonium carbonate washes are formed when cupric sulphate, malachite, cuprammonium sulphate ($CuSO_4$. 4 NH_3. H_2O) and the basic cupric carbonate of Burgundy mixture are dissolved in ammonium carbonate (NH_4HCO_3. NH_4. NH_2CO_2). The washes are very stable, the most stable of the cuprammonium fungicides, only decomposing slowly

on long standing with formation of malachite. When the washes dry on sprayed foliage the copper is deposited as a carbonate.

Cuprammonium sulphate-ammonium carbonate. This wash was introduced by the United States Department of Agriculture in 1890[22] under the name of mixture number 5 but has never been used to any extent in practice as it was not found to possess a lesser toxicity than other cuprammoniums and is not economical to prepare. The formula originally proposed was as follows:

	parts
Ammoniated copper sulphate (Cuprammonium sulphate)	0.21
Ammonium carbonate	0.12
Water to	100

Malachite-ammonium carbonate. This fungicide was introduced by Chester[23] in 1891 as a substitute for the malachite-ammonia wash, but despite manifest advantages has been but little used in practice. Chester's formula is as follows:

	parts
Malachite	0.052–0.058
Ammonium carbonate	0.27 –0.31
Water to	100

The original formula has been modified to some extent by subsequent writers as is shown in table 10.

TABLE 10

Strength of malachite-ammonium carbonate wash and corresponding ratios malachite: ammonium carbonate recommended by different authors

STRENGTH IN MALACHITE	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM CARBONATE}}$
per cent	
0.039	1:6
0.046	1:5.3
0.052	1:5.19
0.058	1:5.34
0.066	1:3.3
0.093	1:5.3

Malachite dissolves fairly readily in ammonium carbonate, carbon dioxid and a little ammonia being evolved during the reaction, but the reaction is not sufficiently rapid nor the conditions under which it takes place such as to permit the preparation of the fungicide as required. A stock solution is necessary and may be conveniently prepared as follows:

[22] United States Department of Agriculture Rept. **1890**: 402.

[23] Delaware Agr. Exp. Sta. Rept. **4**: 71. 1891.

	parts
Malachite	1
Ammonium carbonate (hard)[24]	3
Water	20

Place the malachite in a suitable non-metal vessel, add the ammonium carbonate in small pieces, and then the water. Warm gently and as soon as effervescence begins remove from flame and stir. Let stand a few minutes, place back on flame and continue as before until on warming gently no further effervescence takes place. The vessel should then be closed and set aside until the malachite has completely dissolved, should it not already have done so. The stock solution prepared as above will withstand marked dilution without a further addition of ammonium carbonate being required at least within the range of concentration in copper that I have used, as will be seen from table 11.

TABLE 11

Amount of ammonium carbonate (hard) required to give clear solutions of malachite-ammonium carbonate

STRENGTH IN MALACHITE	AMOUNT AMMONIUM CARBONATE REQUIRED TO GIVE A CLEAR SOLUTION	RATIO $\frac{\text{MALACHITE}}{\text{AMMONIUM CARBONATE}}$	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*		*per cent*
0.50	1.5	1:3	0.476
0.20	0.6	1:3	0.190
0.10	0.3	1:3	0.095
0.05	0.15	1:3	0.047

Copper sulphate and ammonium carbonate. The copper sulphate and ammonium carbonate wash known in American literature as Johnson's mixture has been but little used in practice and is but rarely mentioned by writers on the fungicides. The wash was first described in 1891 by Johnson[25] who proposed the following formula for its preparation:

	parts
Copper sulphate	0.1
Ammonium carbonate (hard)	0.20
or Ammonium carbonate (soft)	0.25
Water to	100

[24] Ammonium carbonate decomposes on exposure to air and in preparing the stock solution the amount of the salt used will have to be increased unless it is in perfectly hard translucent plates. When completely decomposed ammonium carbonate occurs as an opaque powder and when in this condition the amount called for in the formula should be doubled.

[25] Johnson, S. W. Note by the Director. Connecticut Agr. Exp. Sta. Rept. **1890**: 113. 1891.

Johnson's mixture is, next to the copper sulphate and ammonia wash (eau céleste), the most easily prepared of all the cuprammoniums. When ammonium carbonate is added to a strong solution of cupric sulphate a precipitate is first formed accompanied by effervescence due to the liberation of carbon dioxid which on a further addition of ammonium carbonate promptly and completely dissolves even in the cold.

Johnson's mixture forms very stable solutions and, as a glance at table 12 will show, is for equivalent of copper compositionally identical with malachite-ammonium carbonate.

TABLE 12

Amount of ammonium carbonate hard and soft required to give clear solutions of Johnson's mixture and corresponding ratios cupric sulphate: ammonium carbonate

STRENGTH IN CUPRIC SULPHATE	AMOUNT AMMONIUM CARBONATE REQUIRED TO GIVE A CLEAR SOLUTION		RATIO $\frac{\text{CUPRIC SULPHATE}}{\text{AMMONIUM CARBONATE}}$[1]	STRENGTH OF SOLUTION IN NH_3[1]
	Hard	Soft		
per cent	*per cent*	*per cent*		*per cent*
1.00	1.56	2.40	1:1.56	0.495
0.50	0.78	1.20	1:1.56	0.247
0.20	0.31	0.48	1:1.56	0.099
0.10	0.15	0.24	1:1.56	0.049

[1] Refers to solutions prepared with undecomposed ammonium carbonate.

Burgundy mixture-ammonium carbonate. Burgundy mixture may be readily dissolved in ammonium carbonate yielding a cuprammonium very similar to those obtained with malachite, copper sulphate, or cuprammonium sulphate as will be seen by a glance at table 13.

TABLE 13

Amount of ammonium carbonate required to give clear solution of Burgundy mixture-ammonium carbonate and corresponding ratios Burgundy mixture: ammonium carbonate

STRENGTH IN COPPER SULPHATE	AMOUNT AMMONIUM CARBONATE (HARD) REQUIRED TO GIVE A CLEAR SOLUTION	RATIO $\frac{\text{BURGUNDY MIXTURE}}{\text{AMMONIUM CARBONATE}}$	STRENGTH OF SOLUTION IN NH_3
per cent	*per cent*		*per cent*
1.00	1.20	1:1.20	0.380
0.50	0.60	1:1.20	0.190
0.25	0.30	1:1.20	0.095
0.10	0.12	1:1.20	0.038

The data given in table 13 are for a Burgundy mixture in which the ratio copper sulphate sodium carbonate (crys.) was 1:1.84, and the precipitate was separated from the mother liquor before dissolving in the

ammonium carbonate. In practice, however, the precipitate would not need to be separated from the mother liquor, since the salts therein contained are not injurious[26] at the concentrations at which the wash may be employed in practice.

As a result of our study of the properties and preparation of the cuprammonium washes we may conclude:

1. The cuprammonium carbonate washes are the most stable and for strengths in metallic copper of 0.14 per cent or less require less ammonia to give clear solutions than the washes prepared with ammonium hydroxid.

2. The copper salts dissolved in ammonium carbonate yield washes in which the active principle, i.e., the copper is in the form of a carbonate.

3. Metallic copper, malachite, and the basic carbonate of Burgundy mixture form cuprammonium hydrates when dissolved in ammonium hydroxid modified eau céleste requiring per equivalent of copper the least amount of solvent.

4. Cupric sulphate forms with ammonium hydroxid a cuprammonium sulphate which is the least stable of the cuprammonium fungicides, though less ammonia is required to give a clear solution than in the case of the cuprammonium hydrates.

II. RELATIVE TOXICITY OF THE CUPRAMMONIUM WASHES

The cuprammonium washes may be toxic to the sprayed plant: (1) Between the time of application and time of drying; (2) after drying owing to dissolution of the contained copper on weathering; or (3) the injury produced, if any, may be the result of the additive effect of 1 and 2. There are no other possibilities.

A. Effect of the cuprammoniums on plants between the time of application and the time of drying

It will be at once evident that if the cuprammoniums made with ammonium hydroxid owe their toxicity to the presence of ammonia, the toxic action, owing to the rapid dissipation of ammonia in the interim between time of application when its concentration will be highest and time of desiccation when its concentration will be zero, must and can only take place during the drying of the spray. Ammonium carbonate is less volatile than ammonium hydroxid, but nevertheless decomposes readily on exposure to air and has vanished when the washes of which it is a component have dried upon the foliage, hence injury due to the presence of ammonia in ammonium carbonate can also only be produced in the

[26] See p. 257.

TABLE 14

Effect of quick and slow drying on the toxicity of the cuprammonium washes[1] expressed in per cent of injury

VARIETY USED	HOW DRIED	FUNGICIDE USED AND STRENGTH AT WHICH EMPLOYED															
		Copper sulphate-ammonia				Johnson's mixture				Malachite-ammonia				Malachite-ammonium carbonate			
		Per cent Cu				Per cent Cu				Per cent Cu				Per cent Cu			
		0.254	0.127	0.0635	0.0317	0.254	0.127	0.0635	0.0317	0.2874	0.1437	0.0718	0.0359	0.2874	0.1437	0.0718	0.0359
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coleus var. Golden Bedder	Quickly	15	8	0	0	5	2	0	0	32	17	0	0	3	0	0	0
	Slowly	28	19	9	3	15	9	4	2	83	43	17	4	65	34	14	2
Tomato var. Bonny best	Quickly	2	1	0	0	5	0	0	0	40	16	5	0	3	0	0	0
	Slowly	11	6	0	0	39	9	0	0	100	72	30	4	72	55	28	16
Bean var. Dwarf horticultural	Quickly	23	9	7	3	37	27	19	5	40	26	13	4	24	11	10	4
	Slowly	27	13	10	5	46	30	20	8	60	45	24	5	29	16	10	7
Pelargonium varieties	Quickly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Slowly	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] The data presented in the tables of this chapter are the mean values of four duplicate experiments though sometimes more and in a few instances only three have been performed. The plants dried quickly were always dry within two hours while those dried slowly were placed in moist chambers, taken out at the end of six hours and allowed to dry spontaneously. The cuprammoniums were applied as perfectly clear solutions and the reader desirous of ascertaining the exact formula employed in any particular case may readily do so by turning to the appropriate table in the previous chapter.

interim between time of application and time of drying. If the toxic action is due, on the other hand, to soluble copper or ammonium sulphate whenever this salt occurs, then the injury resulting may be produced during the time of drying and also at such subsequent times as the fungicide is wetted by meteoric water. The rate at which the cuprammoniums dry upon sprayed foliage will, it may therefore be presumed, have a very marked effect on the degree of injury produced. And in fact experimental evidence fully confirms the presumption as is shown in table 14 and plates III and IV.

The data presented in the above table show how significant was Audoynaud's recommendation that the copper sulphate and ammonia wash be applied during dry, warm weather and fully justifies Bourcart's[27] statement that scorching "is especially to be feared when eau céleste is applied during moist weather and, consequently, when it dries slowly on the leaves;" but that on the other hand "when the spray is applied during the hot days of summer, this fault disappears entirely, and eau celeste possesses nothing but advantages." The data presented in the table show conclusively that slow drying is much more injurious than quick drying, the difference being very striking indeed in the case of malachite-ammonia and malachite-ammonium carbonate containing 0.28 per cent and 0.14 per cent copper.

While the data presented in table 14 show that whenever a cuprammonium is toxic it is invariably more injurious when dried slowly irrespective of the plant sprayed, it does not give us any very definite information regarding the cause of the deleteriousness of these washes. The data do not support conclusively either the view that ammonia is the toxic agent, or the view that the toxicity is due to soluble copper since were but one of these components the sole cause of the injury produced a certain proportionality would exist differing only in degree in different plants between a given strength of the toxic substance and the resulting injury, but a consideration of table 14 shows that no such relation exists in either case. On the other hand, the data clearly show that ammonium sulphate does not possess any markedly injurious properties. Neither are the malachite washes shown to be less injurious than those made from cupric sulphate and ammonia, a fact worthy of serious consideration in view of the general abandonment of the latter on account of supposed greater toxicity.

But since no individual component of the cuprammonium washes is apparently *per se* the cause of the toxic action produced in the interval between the application of the fungicide and its desiccation it will be

[27] Bourcart, E. Les maladies des plants, 376. Paris. 1910.

necessary for us to study their behavior when applied separately in order to interpret the data given in table 14, and to the action of soluble copper we may well devote attention first.

The only suitable copper salt to use in studying the effect of soluble copper when applied in the form of a spray is cupric sulphate and since the acid radicals of the copper salts are not in themselves injurious[28] the toxicity of cupric sulphate may be considered as due to the copper. Soluble copper is, as is well known, extremely toxic to vegetation and we would, therefore, expect that the injury produced by an application of cupric sulphate would be the greater the slower the spray dried on the foliage, and the data presented in table 15, fully confirm this expectation, and the illustration shown in plate V is no less emphatic.

TABLE 15

Effect of quick and slow drying on the toxicity of cupric sulphate expressed in per cent of injury

VARIETY USED	HOW DRIED	MILLIGRAMS PER CENT COPPER (CU.)[1]									
		508	254	127	63	31	15	7.9	3.9	1.9	0.99
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coleus	Quickly		14	7	3	2	0				
	Slowly		37	18	8	5	4				
Tomato	Quickly	40	18	10	8	4	0	0			
	Slowly	90	67	55	32	16	10	0			
Cauliflower var. Snowball	Quickly			42	39	29	18	15	7	5	0
	Slowly			58	53	35	31	28	15	14	2
Bean	Quickly				50	29	19	0	0	0	
	Slowly				80	56	37	20	8	0	
Oxalis bowei	Quickly			33	19	13	5				
	Slowly			60	48	29	20				
Pelargonium Martha Washington var.	Quickly	20	11	3	0						
	Slowly	60	26	13	5						

[1] The heading, used also in plate viii, means that the percentage strength of the solution is given in terms of milligrams instead of fractions of a gram.

The data presented in table 15 and shown graphically in plate VIII indicate that the tomato, Oxalis, bean and cauliflower are much more sensitive to soluble copper than either the Coleus or the Pelargonium. The tomato, bean, Oxalis and cauliflower respond in a somewhat similar manner to soluble copper, the curves for quick and slow drying being

[28] Bedord, Duke of, and Pickering, S. U. Woburn Experimental Fruit Farm Rept. **11**: 1910.

Clark, J. F. On the toxic properties of some copper compounds with special reference to bordeaux mixture. Bot. Gaz. **33**: 39. 1902.

Hawkins, L. A. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals towards fungus spores. Physiological Researches **1**: 87. 1913–16.

very nearly parallel at the higher concentrations. In the case of the cauliflower which is very sensitive to soluble copper the curves are nearly proximate, while in the case of the tomato, which is much more tolerant, they are distant. The curves for the Oxalis and bean are very similar in character, and resemble those of the tomato, though the bean is more sensitive to soluble copper than the Oxalis and the Oxalis less resistant than the tomato. In the most highly resistant plants studied, the Pelargonium and Coleus, the curves show marked similarity, the toxicity on slow drying increasing a little more rapidly in the former than in the latter.

It will also be noticed that while in the more resistant plants (Coleus, Pelargonium) the toxicity is practically proportional to the concentration in the less resistant plants proportionality ceases to exist as soon as the threshold of toxicity is approached, the decrease being much more rapid than the data for the higher concentrations would lead one to anticipate. The practical importance of these facts will escape no one.

TABLE 16

Effect of slow and quick drying on the toxicity of ammonium hydroxid sp. gr. 90, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION IN NH_3								
		10.29 per cent	5.14 per cent	4.11 per cent	6.57 per cent	2.05 per cent	1.28 per cent	1.02 per cent	0.51 per cent	0.25 per cent
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Coleus	Quickly				18			10	7	0
	Slowly				76			25	8	2
Tomato	Quickly			50		29		20	12	5
	Slowly			70		50		46	34	22
Cauliflower	Quickly	60	22		10		0		0	0
	Slowly	75	33		21		5		3	0
Bean	Quickly			23		16		2		
	Slowly			47		35		10		

The toxicity of ammonium hydroxid has been considered high by all writers who have held the tenet that the toxicity of the copper and ammonia washes was due to the ammonium hydroxid though Millardet poured, he tells us, strong ammonium hydroxid on the foliage of the grape without producing injury, a result strongly contradicting the prevailing view, and the data presented in table 16 are not on analysis favorable to it.

A glance at the table reveals that like the cuprammoniums and cupric sulphate ammonium hydroxid even though highly volatile is much more toxic when dried slowly and the illustration given in plate VI shows very strikingly what marked differences may be obtained. The data also show that, of all the plants used, the tomato is the most sensitive

to ammonium hydroxid while the Coleus, bean and cauliflower follow in increasing order of resistance. Now if ammonium hydroxid is primarily the cause of the toxicity of the cuprammoniums of which it is a component part the relative resistance of the plants when sprayed with the copper sulphate and ammonia wash or the malachite-ammonia wash should remain unchanged. In reality we find that when the former is employed the sensitiveness of the plants is in decreasing order as follows: Tomato, bean, Coleus;[29] and when the latter is used that it is as follows: Tomato, Coleus, bean. The evidence is therefore clear that ammonium hydroxid is not the primary cause of the toxicity of the cuprammoniums.

The toxicity of ammonium hydroxid is of course due to the ammonia (NH_3) it contains and it would therefore be expected that ammonium carbonate which has been used in the preparation of cuprammonium washes would also prove injurious since while less volatile and alkaline than the former it nevertheless decomposes rapidly on exposure to the air with liberation of ammonia and carbon dioxid. And in fact experimentation shows ammonium carbonate to be much more toxic than usually supposed as will appear from a consideration of table 17.

TABLE 17

Effect of slow and quick drying on the toxicity of ammonium carbonate, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION IN NH_3			
		1.90 per cent	0.95 per cent	0.47 per cent	0.23 per cent
		per cent	*per cent*	*per cent*	*per cent*
Coleus	Quickly	40	2	0	0
	Slowly	100	81	0	0
Tomato	Quickly	40	15	5	0
	Slowly	100	70	50	20
Cauliflower	Quickly	75	29	10	7
	Slowly	92	42	13	6
Bean	Quickly		1	0	0
	Slowly		8	0	0

The data presented in table 17 and more strikingly illustrated in the graphs (plates IX and X) show that within the limits of toxicity ammonium carbonate is much more injurious per equivalent of ammonia than ammonium hydroxid and it is rather difficult to understand how it could ever have come to be considered less noxious than the former. As would be expected from the nature of the salt, the toxicity of ammonium car-

[29] The cauliflower was not used in the experiments on the toxicity of the cuprammoniums.

bonate when dried slowly is much more marked than when it is dried quickly. The illustration (plate VII, fig. 1), will show what striking effects may be obtained.

The data show that of all the plants studied the tomato is the most sensitive to ammonium carbonate, the susceptibility of the other plants being in order of increasing resistance as follows: Coleus, cauliflower, bean. Similarly in the case of ammonium hydroxid, the relative susceptibility of the plants is in increasing order as follows: Tomato, Coleus, bean, cauliflower. The relative toxicity of ammonium hydroxid and ammonium carbonate is clearly shown in plates IX and X. An inspection of the graphs on these plates shows that the toxicity of ammonium carbonate, whether dried quickly or slowly, increases much more rapidly with the concentration than does the toxicity of ammonium hydroxid, the curves for the former indicating proportionality between concentration and toxicity while those for the latter resemble those given by copper sulphate. A consideration of plate IX shows that ammonium carbonate is more toxic to the bean and cauliflower irrespective of the rate at which dried than ammonium hydroxid. From plate X, on the other hand, we gather that in the case of the tomato ammonium carbonate is less toxic between 0 and 0.95 per cent ammonia than ammonium hydroxid when dried quickly, but is invariably more toxic when dried slowly; in the case of the Coleus ammonium carbonate dried quickly is less toxic between 0 and 1.02 per cent ammonia than ammonium hydroxid and, when dried slowly, less toxic between 0 and 0.51 per cent.

Ammonium sulphate has been considered by certain writers as the toxic agent of the cuprammoniums in which it occurs and the copper sulphate and ammonia wash has suffered relegation in consequence of this view. Pearson,[30] however, at an early date impugned this belief for he found that the mother liquor of a strong copper sulphate ammonia wash caused no injury to the grape vine, the strawberry and "various other vegetables." A glance at table 18 will show that ammonium sulphate can not possibly be the cause of the toxicity of the cuprammoniums in which it occurs, since the amount of ammonium sulphate formed could not exceed 0.53 gram for every gram of cupric sulphate employed. But if ammonium sulphate can not be the cause of the toxicity of the copper sulphate and ammonia wash or Johnson's mixture, the data presented in the table show that it possesses a peculiarity to which we may well devote a moment's attention.

[30] Pearson, A. N. *In* Report on the experiments made in 1888 in the treatment of the downy mildew and blackrot of the grape vine. U. S. Dept. Agr., Sec. Veg. Path. Bul. **10: 18. 1889.**

TABLE 18

Effect of slow and quick drying on the toxicity of ammonium sulphate, expressed in per cent of injury

VARIETY USED	HOW DRIED	STRENGTH OF SOLUTION			
		4 per cent	2 per cent	1 per cent	0.5 per cent
		per cent	*per cent*	*per cent*	*per cent*
Coleus	Quickly	91	66	24	0
	Slowly	24	19	0	0
Bean	Quickly	63	28	16	0
	Slowly	25	4	1	0
Tomato	Quickly	73	34	8	0
	Slowly	50	22	6	0

TABLE 19

Effects on tomato, Coleus and bean of the slowly dried cuprammoniums and their components at the strengths at which they occur in the washes, expressed in per cent of injury

FUNGICIDE USED	AMOUNT OF COPPER IN SOLUTION	TOMATO				COLEUS				BEAN			
		Cuprammonium	Cuprie sulphate = soluble copper	Ammonia	Ammonium sulphate	Cuprammonium	Cuprie sulphate = soluble copper	Ammonia	Ammonium sulphate	Cuprammonium	Cuprie sulphate = soluble copper	Ammonia	Ammonium sulphate
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Copper sulphate and ammonia	0.254	11	67	27	0	28	37	3	0	27	100	0	0
	0.127	6	55	22	0	19	18	1	0	13	100	0	0
	0.063	0	32	15	0	9	8	0	0	10	80	0	0
	0.031	0	16	13	0	3	5	0	0	5	56	0	0
Johnson's mixture	0.254	39	67	34	0	15	37	0	0	46	100	0	0
	0.127	9	55	10	0	9	18	0	0	30	100	0	0
	0.063	0	32	0	0	4	8	0	0	20	80	0	0
	0.031	0	16	0	0	2	5	0	0	8	56	0	0
Malachite-Ammonia	0.287	100	67	91		83	37	100		60	100	51	
	0.143	72	55	64		43	18	100		45	100	45	
	0.071	34	32	50		17	8	49		24	80	28	
	0.035	4	16	38		4	5	14		5	56	0	
Malachite-Ammonium carbonate	0.287	72	67	50		65	37	39		29	100	0	
	0.143	55	55	18		34	18	0		16	100	0	
	0.071	28	32	0		14	8	0		10	80	0	
	0.035	16	16	0		2	5	0		7	56	0	

Ammonium sulphate is more toxic when dried quickly than when dried slowly, behaving in this respect exactly the reverse of the other components of the cuprammoniums all of which are much more toxic when dried slowly. The injury produced by quick drying may be very marked and the illustration, plate VII, fig. 2, will add but emphasis to the striking figures in the table. The explanation of this peculiarity is that ammonium sulphate is slightly hygroscopic and when dried quickly withdraws water from the leaf which wilts and dies, but that when dried slowly equilibrium between the cells of the epidermis and the solution is reached by the time drying begins.

The plant most seriously injured by quick drying is the Coleus, then follows the bean, the tomato being but little more seriously affected by quick drying than by slow drying, the data for 1 per cent solutions being in this respect particularly instructive. It would appear therefore that the cuticles of the Coleus and bean are not readily permeated by ammonium sulphate while that of the tomato is easily penetrated which facts are of considerable interest since slow drying which properly measures the toxicity of the salt shows that the most readily penetrated leaf is also the most susceptible.

Having concluded our study of the components of the cuprammoniums we are in a position to determine the nature of their rôle in the toxicity of these washes. In order to simplify our study I have placed *en regard* in table 19 the percentage injuries produced on the one hand by the several cuprammoniums and on the other the injury that would have followed the use of the components at the corresponding strengths at which they occur in the washes, the data being in all cases for slow drying.

The data presented in the table confirm in large measure the view of Millardet that soluble copper is the cause of the injury produced by the cuprammoniums though it would also appear that ammonia becomes toxic when its concentration exceeds an amount that may or may not be considerably in excess of that normally tolerated by the plant concerned. For instance in the case of the Coleus, malachite-ammonium carbonate containing 0.287 per cent copper, is more toxic than the equivalent strength of soluble copper or ammonia whence it must be concluded that the injury is due to combined action of the two; and it would appear from the behavior of this wash at other strengths that the presence of ammonia has in all cases even when itself not apparently toxic, increased the toxicity of the copper. The malachite-ammonium carbonate wash also shows evidence of an additive effect except at the lowest concentration used. On the other hand, Johnson's mixture shows that the toxicity of soluble copper is reduced by its presence despite the fact that the concentration of ammonia is very nearly the same as in malachite-ammo-

nium carbonate; but in the case of the copper sulphate and ammonia wash the evidence is conflicting. When we come to consider the tomato we find that the presence of ammonia reduces the toxicity of soluble copper in the case of Johnson's mixture and the copper sulphate and ammonia wash, but that in the malachite-ammonia wash its toxicity is increased except at the lowest concentration used. In the case of malachite-ammonium carbonate we have the toxicity of soluble copper increased at the highest concentration (0.287 per cent copper) not changed at 0.143 per cent copper and 0.035 per cent copper and reduced at 0.071 per cent copper.

Finally in the case of the bean we find that the presence of ammonia has in all cases reduced the toxicity of soluble copper.

The evidence is therefore in favor of the view that the presence of ammonia is beneficial and not injurious as too commonly supposed though it should be noted that while this beneficent action of ammonia is sufficiently general to be considered established and is independent of the rate at which the cuprammoniums decompose, the data nevertheless clearly show that cuprammonium sulphate, the most unstable of the washes, is also, all things considered, less injurious than cuprammonium hydrate and carbonates which are very stable. Soluble copper must therefore be the major cause of the toxicity of the cuprammoniums in the interim between application and desiccation.

B. Effect of the cuprammoniums after they have dried upon the plant and are subject to the action of the weather

After the cuprammoniums have dried upon the plant there will be present on the leaf only a copper carbonate or hydrate in the case of the washes prepared from malachite, but in the case of those prepared with cupric sulphate (copper sulphate and ammonia wash, Johnson's mixture) ammonium sulphate not in excess of one-half of the cupric sulphate employed will also be present, and in the case of the washes prepared from Burgundy mixture we will have besides the copper salt both sodium bicarbonate and sodium sulphate present, the former to the extent of 0.41 gram for every gram of cupric sulphate taken, the latter to the extent of 0.56 gram for every gram of cupric sulphate used.[31] In two instances, therefore, the dissolution of the copper in meteoric waters is not affected by the presence of a foreign substance, in two instances the foreign substances (sodium sulphate and bicarbonate) are neither toxic

[31] The calculations are based on the formula (vide Bedford, Duke of, and Pickering, S. U. loc. cit., p. 86). $5\ CuSO_4\ 5\ H_2O + 8\ Na_2CO_3 + 10\ H_2O = 2\ CuCO_3,\ 3\ Cu(OH)_2 + 6\ NaHCO_3 + 5\ Na_2SO_4 + 105\ H_2O$.

at the strengths at which they are found in the washes or solvents of the copper salt, and in two instances the action of meteoric waters is heightened by the presence of ammonium sulphate which is a solvent of insoluble copper salts but non-toxic itself at the strength at which it occurs in the washes. It is, therefore, clear that the toxicity of the cuprammonium washes after they have dried upon the leaf can only be due to the presence of soluble copper. Whence it will only be necessary, in order to determine the magnitude of this toxicity, to ascertain the degree of solubility of the copper in the several cuprammoniums as compared with that of a soluble copper salt, and, as already indicated, cupric sulphate is the only common inorganic salt of copper that satisfies the conditions under the experimental methods employed. It is desirable in testing the relative solubility of cupric sulphate and of the copper salt of the dried cuprammoniums that an organism sensitive to soluble copper be employed as indicator. The conidia of *Plasmopara viticola* are, as is well known, extremely sensitive to soluble copper[32] and are admirably adapted to this purpose and were employed in obtaining the data presented in table 20. The data were obtained by spraying microscopic slides after the manner described by Reddick and Wallace[33] with the solutions to be tested, allowing them to dry spontaneously at room temperature and putting in service not earlier than twenty-four hours after the fungicides had dried. In making a test the spores were washed with distilled water into a beaker from leaves just freshly gathered. Small drops of water with spores in suspension were taken from the beaker, and placed on the slides which were then incubated at or near the optimum temperature for indirect germination.[34] Simultaneously a witness was always prepared so that the vitality of the spores could be properly judged and no experiment was considered in which the germination in the witness proved low. Finally it should be noted that the data given in table 20 are the mean of five experiments except in the case of the malachite-ammonia wash with which only two tests were made.

The data show that the presence of ammonium sulphate increases the toxicity of a wash, the unit copper in copper sulphate and ammonium carbonate being more toxic than the unit copper in malachite-ammonium

[32] Millardet, A. and Gayon, U. Traitement du mildiou par le mélange de sulfate de cuivre et de chaux. Journ. agr. prat. **2**: 709. 1885.

Wüthrich, E. Ueber die Einwirkung von Metallsalzen und Säuren auf die Keimfähigkeit der Sporen einiger des Verbreitetsten parasitischen Pilze unserer Kulturpflanzen. Zeit. Pflanzenkr. **2**: 16–31, 81–94. 1892.

[33] Reddick, D. and Wallace, E. On a laboratory method of determining the fungicidal value of a spray mixture or solution. Science n. s. **31**: 798. 1910.

[34] Melhus, I. E. Germination and infection with the fungus of the late blight of potato. Wisconsin Agr. Exp. Sta. Tech. Bul. **37**: 36. 1915.

carbonate. It will be also noticed that the highest toxic value for the unit copper occurs in the copper sulphate-ammonia wash, though on account of the presence of ammonium sulphate it is impossible to tell to what extent this high value is due to the presence of this solvent, to what extent to the basic cupric sulphate. Judging from the increased toxicity of cupric carbonate in the presence of ammonium sulphate it would seem that the high toxic value of the unit copper in basic cupric sulphate is due to the presence of ammonium sulphate. However this may be, the table shows conclusively that the unit copper in the cuprammonium carbonate washes has a lower toxic value than in a cuprammonium hydrate or sulphate[35] wash.

TABLE 20

Relative toxicity of the cuprammonium washes and cupric sulphate to the spores of Plasmopara viticola

FUNGICIDE USED	LETHAL STRENGTH	SOLUBILITY OF COPPER
	per cent Cu	*Relative numbers*
Malachite-ammonia	0.0033	58.13
Malachite-ammonium carbonate	0.0057	33.33
Copper sulphate-ammonia	0.0027	70.42
Copper sulphate-ammonium carbonate	0.0035	54.34
Cupric sulphate	0.0019	100.00

Knowing the toxicity of cupric sulphate to any given higher plant it may be calculated readily from the data given in table 21 at what strength the cuprammoniums would have to be used in order to prove uninjurious after they had dried on the foliage and are wetted by meteoric water. Taking the plants used in the experiments with cupric sulphate[36] we arrive at the results shown in table 21, the data for the cupric sulphate being obtained from table 15, either directly or by extrapolation.

The data presented in the table show the strength at which the several cuprammoniums could be applied to foliage without producing injury after drying due to dissolution of the copper in meteoric waters. The question that we must now consider is whether these strengths are superior to or smaller than those at which injury occurs between time of application and time of drying. If inferior then the maximum strength at which the cuprammoniums can be used will be governed by the solubility of the copper after drying; if superior, then the degree of injury

[35] There is no reason for supposing that modified eau céleste or Burgundy mixture-ammonium carbonate would possess a toxicity materially different from their prototypes mentioned in the table.

[36] See p. 251.

produced while drying will limit the strength at which these fungicides may be used safely. The data presented in table 22 will permit us to answer the question raised.

The data show that in the case of all four fungicides the degree of tolerance of the dried fungicides must limit the strength at which they can be used.

TABLE 21

Calculated tolerance of plants to dried cuprammoniums

PLANT EMPLOYED	CUPRIC SULPHATE DRIED SLOWLY NON-TOXIC	MAXIMUM STRENGTH AT WHICH CUPRAMMONIUMS COULD BE USED			
		Malachite-ammonia	Malachite-ammonium carbonate	Copper sulphate-ammonia	Copper sulphate-ammonium carbonate
	per cent Cu	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*
Coleus	0.0075	0.0119	0.0225	0.0106	0.0138
Tomato	0.0075	0.0119	0.0225	0.0106	0.0138
Cauliflower	0.0007	0.00120	0.0021	0.00099	0.00128
Pelargonium	0.0317	0.0545	0.0951	0.0450	0.0583
Bean	0.0019	0.0032	0.0057	0.0026	0.0034
Oxalis	0.0079	0.01358	0.0237	0.0112	0.0145

TABLE 22

Amount of copper tolerated in the several cuprammoniums during and after drying

FUNGICIDE USED	COLEUS		TOMATO		BEAN	
	Copper tolerated		Copper tolerated		Copper tolerated	
	During slow drying	After drying	During slow drying	After drying	During slow drying	After drying
	per cent Cu	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*	*per cent Cu*
Malachite-ammonia	0.0298	0.01358	0.0327	0.01358	0.0287	0.0032
Malachite-ammonium carbonate	0.0375	0.0237	0.0287	0.0237	0.0183	0.0057
Copper sulphate-ammonia	0.0228	0.0112	0.0635	0.0112	0.0152	0.0026
Copper sulphate-ammonium carbonate	0.0254	0.0145	0.0635	0.0145	0.0228	0.0034

C. Injury by additive effect

As we have seen the cuprammoniums may produce injury during drying and after drying due to dissolution of the copper in meteoric waters. When the injuries produced by these sources is sufficiently distant, their mutual effects remain distinct, but when they occur nearly simultaneously an injury greater than that due to the sum of the effects

of the injury produced during drying and after drying is to be anticipated since as Schander[37] and Barker and Gimingham[38] have pointed out soluble copper is more injurious to recently than to remotely injured leaves. The data presented in table 22 show clearly that if injury is produced during drying, injury will also follow wetting with meteoric water. The converse is not, however, necessarily true, since the rate of drying markedly affects, as we have seen, the degree of tolerance of copper.

III. PRACTICAL CONSIDERATIONS

If we consider the strength at which the cuprammoniums are or have been used in practice, we will find that these fungicides are, or have been, as will be seen from table 23, applied at a strength in copper ranging from 7.2 to 47 times the lethal concentration for *Plasmopara viticola*.

TABLE 23

Strength at which the cuprammoniums have been most commonly employed in practice and lethal concentration of the same for Plasmopara viticola

FUNGICIDE USED	A	B	RATIO $\frac{A}{B}$
	Strength at which applied	Lethal concentration	
	per cent Cu	*per cent Cu*	
Malachite-ammonia	0.0493	0.0033	1:14.9
Malachite-ammonium carbonate	0.0264	0.0057	1:4.63
Copper sulphate-ammonia	0.0270	0.0027	1:47.03
Copper sulphate-ammonium carbonate	0.0254	0.0035	1:7.25

Now since the cuprammoniums may all be considered equally adhesive, as regards resistance to mechanical shock, because they form precipitates composed of particles of nearly like size, the concentration at which they are applied should bear a definite relation either to their efficiency or effectiveness.[39] But an inspection of table 23 will immediately show that no relation exists between lethal concentration and strength of application whether we take as our criterion efficiency or effectiveness. The

[37] Schander, R. Über die physiologische Wirkung der Kupfervitriol Kalkbrühe. Landw. Jahrb. **33**: —. 1904.

[38] Barker, B. T. P. and Gimingham, C. T. The action of Bordeaux mixture on plants. Ann. Appl. Biology **1**: 11 *et seq.* 1914.

[39] The efficiency of a fungicide depends both on the solubility of the copper and its toxicity when in solution. Usually, though not necessarily always, solubility is a measure of toxicity and conversely. Effectiveness depends on the power of a fungicide to withstand weathering, that is, to possess adhesive properties, while at the same time yielding sufficient soluble copper to give protection from a specific organism or organisms.

formulae in use should therefore be amended so as to give a numerical relation between lethal concentration and strength of application.

Let us first of all determine the factor required to give maximum effectiveness.

Since we have been unable to assign a value to this factor from the data presented in table 24, it will be necessary for us to determine it indirectly and this we can do from our knowledge of Bordeaux mixture. According to a recent French enquiry[40] a 2 per cent Bordeaux mixture is necessary to give adequate protection from *Plasmopara viticola* in years favorable to the development of this parasite though in years when infestation is not severe 1 per cent mixtures meet more or less satisfactorily the requirements of practice. In the United States, on the other hand, both *Plasmopara viticola* and *Phytophthora infestans* may be satisfactorily held in check by 1 per cent Bordeaux mixtures though 1.25 per cent mixtures are also commonly employed. We may therefore in all propriety take a 1 per cent Bordeaux mixture for our standard of comparison.

Now Bordeaux mixture 1:1 is toxic to both *Plasmopara viticola* and *Phytophthora infestans*[41] at 0.0039 per cent copper which gives us a factor of 64 as the requirement of practice for adequate protection. Accepting this factor of 64 for Bordeaux mixture the factors necessary to apply to the cuprammoniums, due regard being taken of the relative efficiency of the unit copper, in order to obtain satisfactory protection would then be as indicated in table 24.

TABLE 24

Factors by which the lethal concentrations of the several cuprammoniums must be multiplied in order to obtain adequate protection

FUNGICIDE USED	LETHAL CONCENTRATION	FACTOR
	per cent Cu	
Bordeaux mixture 1:1	0.0039	64
Malachite-ammonia	0.0033	53.7
Malachite-ammonium carbonate	0.0057	93.4
Copper sulphate-ammonia	0.0027	44.1
Copper sulphate-ammonium carbonate	0.0035	56.9

From the data given in table 24 one can readily determine the strength at which the cuprammoniums should be used in practice in order to obtain a protection substantially equivalent to that given by Bordeaux mixture, and when the data so obtained are compared with the strengths

[40] Capus, J. Les traitements du mildiou. Rev. de vit. **44**: 302. 1916.

[41] Wisconsin Agr. Exp. Sta. Tech. Bul. **37**: 36. 1915.

employed in practice, as in the following table, we find that the calculated strengths are from four to twenty times greater than those that have been actually used except in the case of the copper sulphate and ammonia wash when the agreement is close. But since the cuprammoniums have not afforded, at the strengths used in practice, protection commensurate with Bordeaux mixture and we are now in a position to understand why they have not, the formulae employed should be emended so as to approach the calculated values indicated. But the actual strengths employed will be determined by the tolerance of the plant sprayed to the fungicide in the interim between application and desiccation, and to the solubility of the dried wash in meteoric waters. We will first of all, consider the latter case.

The dried cuprammoniums must not, of course, yield on being wetted more soluble copper than the sprayed plant will stand. Let us accept

TABLE 25

Strengths at which the cuprammoniums are used in practice and strengths at which they should be employed in order to give protection equivalent to Bordeaux mixture

FUNGICIDE USED	STRENGTH USED IN PRACTICE	STRENGTH EQUIVALENT TO 1 PER CENT BORDEAUX MIXTURE
	per cent Cu	*per cent Cu*
Malachite-ammonia	0.0493	0.1772
Malachite-ammonium carbonate	0.0264	0.5323
Copper sulphate-ammonia	0.1270	0.1190
Copper sulphate-ammonium carbonate	0.0254	0.1991

for the sake of concreteness, the value for the tomato 0.0075 per cent copper. This percentage of metallic copper is yielded by a 0.045 per cent copper sulphate and ammonia wash under laboratory conditions but the tolerance of the tomato under field conditions may be safely placed at 0.125 per cent, since rains even of moderate intensity will carry away appreciable amounts of the fungicide. And since in the other cuprammoniums, as we have seen, the copper is less soluble than in the copper sulphate and ammonia wash, we may safely use them at the same relative concentration which would then give us the following as the permissible strengths at which they can be applied without injury from soluble copper resulting to a plant tolerating 0.0075 per cent soluble copper. As will appear from a consideration of table 26, the calculated values for soluble copper tolerated are lower than those obtaining in practice for the malachite-ammonia and copper sulphate-ammonia washes and higher in the case of the two cuprammonium carbonate washes.

The data presented in table 26 further show that it is impossible to apply the cuprammoniums at strengths equivalent in effectiveness to 1 per cent Bordeaux mixture when the plants sprayed will not tolerate more than 0.0075 per cent soluble copper since in order to obtain equivalence the plant sprayed would have to tolerate 0.0158 per cent soluble copper.

We have admitted that, under the conditions of practice plants will tolerate cuprammoniums 2.8 times stronger than tolerated under critical conditions. It remains now to be seen whether cuprammoniums of this strength can be applied without injury resulting during drying. The data presented in table 27 show that in the case of the tomato under the conditions of quick drying, all the cuprammoniums except the malachite-ammonia wash can be used at the required concentration, but that under the conditions of slow drying malachite-ammonia and malachite-ammonium carbonate, the latter particularly, are toxic at a lower concentration than that demanded. In the case of the Coleus we find quick

TABLE 26

Strengths at which the cuprammoniums may be used without an injurious amount of soluble copper forming on wetting with meteoric water, a plant resistant to 0.0075 per cent soluble copper being presupposed

FUNGICIDE USED	STRENGTH TOLERATED	STRENGTH USED IN PRACTICE
	per cent Cu	*per cent Cu*
Malachite-ammonia	0.0386	0.0493
Malachite-ammonium carbonate	0.0668	0.0264
Copper sulphate-ammonia	0.0317	0.127
Copper sulphate-ammonium carbonate	0.0410	0.0254

drying permits the use in every case of stronger solutions than tolerated in the dried wash, while the reverse is the case if slow drying is permitted. In the case of the bean the plant will tolerate stronger drying than dried washes. In the case of the tomato and Coleus the washes can all be used at 11.7 times their toxic concentration to *Plasmopara viticola* when dried quickly but in the case of the bean they can be applied at only 3.3 times their lethal concentration with safety, no matter how slowly or quickly the washes are dried. It seems, therefore, clear that the cuprammoniums can not be considered as effective as Bordeaux mixture for the control of parasitic organisms which do not require a concentration in soluble copper greater than that yielded by the latter. We have now to consider the cuprammoniums from the point of view of efficiency.

The unit copper in the cuprammoniums has generally been considered more efficient than the unit copper in Bordeaux mixture, and this opinion is undoubtedly well grounded when the cuprammoniums which are practically neutral as soon as dry are compared with alkaline Bordeaux mix-

tures in which the copper is without action during the time required to reach neutrality; but when compared with neutralized or neutral Bordeaux mixture at the lethal strengths to *Plasmopara viticola* the difference in favor of the cuprammoniums is indeed small. In the most efficient wash (copper sulphate and ammonia) the unit copper has a value only 1.44 times that of Bordeaux mixture and in the least efficient (malachite-ammonium carbonate) it is 1.46 times less active. But in fungi resistant to copper the unit copper in the cuprammoniums may be manifold that of Bordeaux mixture, the highest values being given by the copper sulphate and ammonia wash. For instance in the case of the uredospores of *Puccinia Antirrhini* the efficiency of the unit copper in the copper sulphate-ammonia wash is very much greater not to say infinitely greater than the unit copper in Bordeaux mixture since it would appear that the latter is non toxic at all concentrations.[42]

TABLE 27

Amount of copper tolerated in the cuprammonium washes during drying by the tomato, Coleus and bean

FUNGICIDE USED	TOMATO			COLEUS			BEAN		
	Strength of dried wash tolerated	Strength tolerated		Strength of dried wash tolerated	Strength tolerated		Strength of dried wash tolerated	Strength tolerated	
		Wash dried quickly	Wash dried slowly		Wash dried quickly	Wash dried slowly		Wash dried quickly	Wash dried slowly
	per cent Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu	*per cent* Cu
Malachite-ammonia....	0.0386	0.0359	0.0327	0.0386	0.0718	0.0298	0.0089	0.0301	0.0287
Malachite-ammonium carbonate.....	0.0668	0.1437	0.0287	0.0668	0.1437	0.0315	0.0154	0.0229	0.0183
Copper sulphate-ammonia.....	0.0317	0.0635	0.0635	0.0317	0.0635	0.0228	0.0073	0.0203	0.0152
Copper sulphate-ammonium carbonate...	0.0410	0.127	0.0635	0.0410	0.0635	0.0254	0.0101	0.0279	0.0228

It is therefore clear that in the control of parasitic endophytes the spores of which are highly resistant to soluble copper, the cuprammoniums may be of very considerable value provided the plant to be sprayed will withstand the concentration demanded. But except in those cases where Bordeaux mixture is non toxic to the parasite from which protection is sought it does not seem to me that the cuprammoniums as a class or any one cuprammonium in particular possess merits sufficient to warrant

[42] Doran, W. L. Controlling snapdragon rust. Value of copper and sulphur. Florists' Exchange **43**: 501. 1917.

their employment in practice, especially since the quality of inconspicuousness can be obtained quite readily with Bordeaux mixtures 1: alkalinity Bordeaux mixture 1: alkalinity is no more conspicuous than the copper sulphate and ammonia wash when the same amount of copper is applied per square meter in both cases but since the latter usually wets the foliage better than the former it appears to be less highly colored, due to the fact that the copper is spread over a larger surface. Whenever Bordeaux mixture 1: alkalinity does not wet the foliage sufficiently to offer the proper degree of inconspicuousness, the wetting power of the mixture may be increased by the addition of an infusion of quillaia (i.e., saponin) or a small amount of casein. It is therefore not necessary to resort to a cuprammonium when an inconspicuous copper fungicide is required.

SUMMARY

1. The cuprammoniums met with in practice belong chemically in one or the other of the following groups: (1) cuprammonium sulphate; (2) cuprammonium hydrate, and (3) cuprammonium carbonate.

2. When decomposed by drying cuprammonium sulphate deposits the copper as a basic sulphate, cuprammonium hydrate yields a copper hydrate, and cuprammonium carbonate a copper carbonate.

3. Cuprammonium sulphate is very unstable; cuprammonium hydrate and carbonate very stable.

4. The cuprammoniums are more toxic when slowly than when quickly dried.

5. The toxicity of the cuprammoniums during drying and on weathering is due to soluble copper.

6. The copper sulphate and ammonia wash and Johnson's mixture are less injurious than the malachite washes.

7. The cuprammonium washes are more efficient and effective than Bordeaux mixture when large amounts of soluble copper are required to give protection.

8. The cuprammonium washes are less effective than Bordeaux mixture when small amounts of soluble copper suffice to give protection but with the exception of the malachite-ammonium carbonate wash are slightly more efficient.

9. The relative efficiency of the unit copper in the cuprammoniums is in decreasing order as follows: Copper sulphate-ammonia, malachite-ammonia, copper sulphate-ammonium carbonate, malachite-ammonium carbonate.

10. The cuprammoniums may be used at 11.7 times their lethal con-

centration for *Plasmopara viticola* on plants not affected by 0.0075 per cent soluble copper.

11. The cuprammoniums are of limited practical applicability and should not be used in lieu of Bordeaux mixture whenever the latter yields sufficient soluble copper to give protection.

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DESCRIPTION OF PLATES

PLATE III

Effect of the malachite-ammonium carbonate wash containing 0.28 per cent Cu on the tomato var. Bonny best. Plants on the left dried quickly, plants on the right dried slowly. Photograph taken twenty-four hours after the fungicide was applied.

PLATE IV

Effect of the malachite-ammonia wash containing 0.28 per cent Cu on the tomato var. Bonny best. Plants on the left dried quickly, plants on the right dried slowly. Photograph taken twenty-four hours after wash was applied.

PLATE V

Tomato var. Bonny best photographed twenty-four hours after being sprayed with 1 per cent cupric sulphate. Plants on the left dried quickly, plants on the right dried slowly. The plants dried quickly showed slight scorching of young leaflets at the time of making the photograph but the injury was not sufficiently marked to show in the plate; the plants dried slowly were, on the other hand, very seriously injured all the leaves being withered and flaccid.

PLATE VI

Effect of ammonium hydroxid containing 4.11 per cent ammonia on the tomato var. Bonny best. Plants showing no apparent injury dried quickly, withered plants dried slowly. Photograph taken twenty-four hours after treatment.

PLATE VII

FIG. 1. Effect of ammonium carbonate containing 0.95 per cent ammonia on the Coleus var. Golden bedder. Plant on the right dried quickly, plant on the left dried slowly. The photograph was taken forty-eight hours after the salt was sprayed on the plants.

Fig. 2. Effect of a 2 per cent solution of ammonium sulphate on the bean var. Dwarf horticultural. Plant on the left dried slowly, plant on the right dried quickly. Photograph taken forty-eight hours after treatment.

PLATE VIII

Graphs showing the relative toxicity of quickly and slowly dried solutions of cupric sulphate to the Coleus, tomato, Oxalis, Perlargonium, bean and cauliflower.

PLATE IX

Graphs showing relative toxicity of quickly and slowly dried solutions of ammonium hydroxid and ammonium carbonate to the bean and cauliflower.

PLATE X

Graphs showing relative toxicity of quickly and slowly dried solutions of ammonium hydroxid and ammonium carbonate to the tomato and Coleus.

O. B. PHOTO

BUTLER: THE CUPRAMMONIUM WASHES

O. B. PHOTO

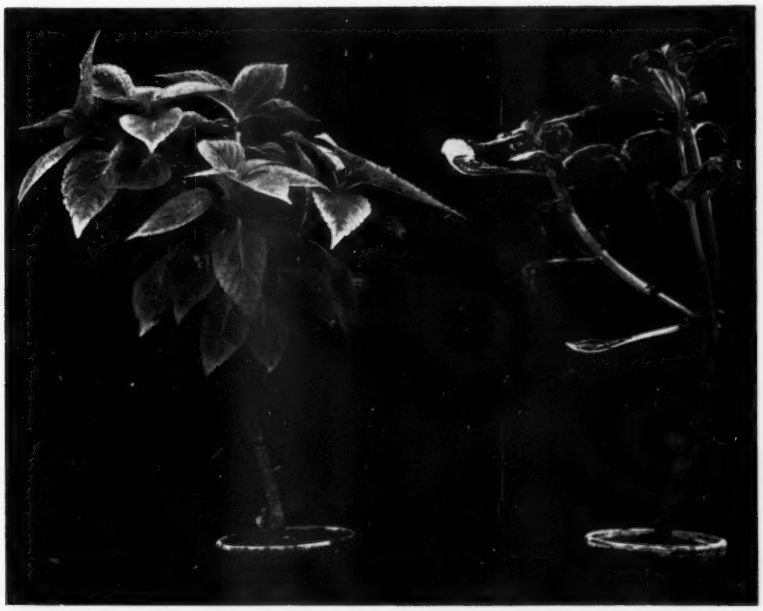
BUTLER: THE CUPRAMMONIUM WASHES

H. B. PHOTO.

BUTLER: THE CUPRAMMONIUM WASHES

O. B PHOTO.

BUTLER: THE CUPRAMMONIUM WASHES



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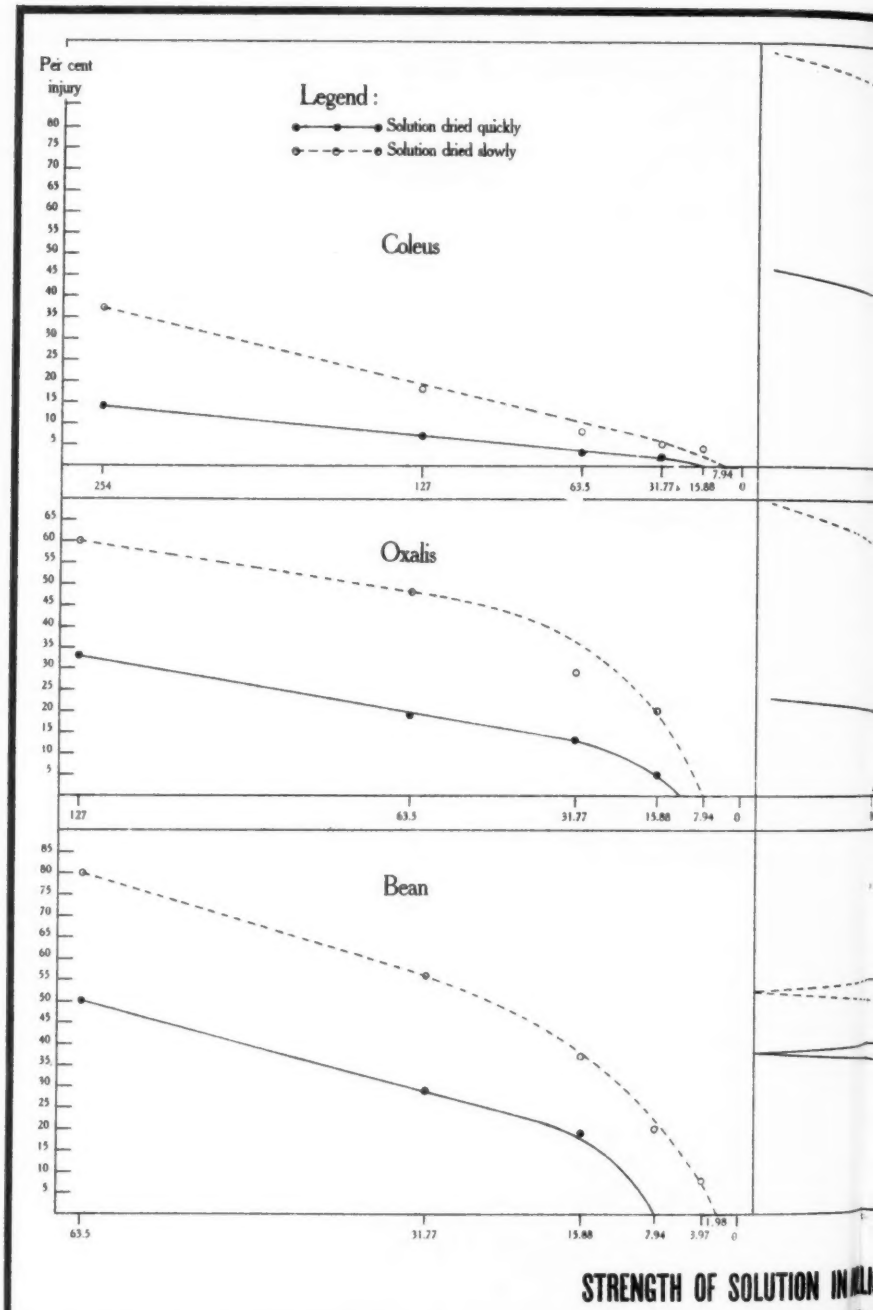
BUTLER: THE CUPRAMMONIUM WASHES


Per cent injury
Legend :
Solution dried quickly
Solution dried slowly
Coleus
Oxalis
Bean
STRENGTH OF SOLUTION IN

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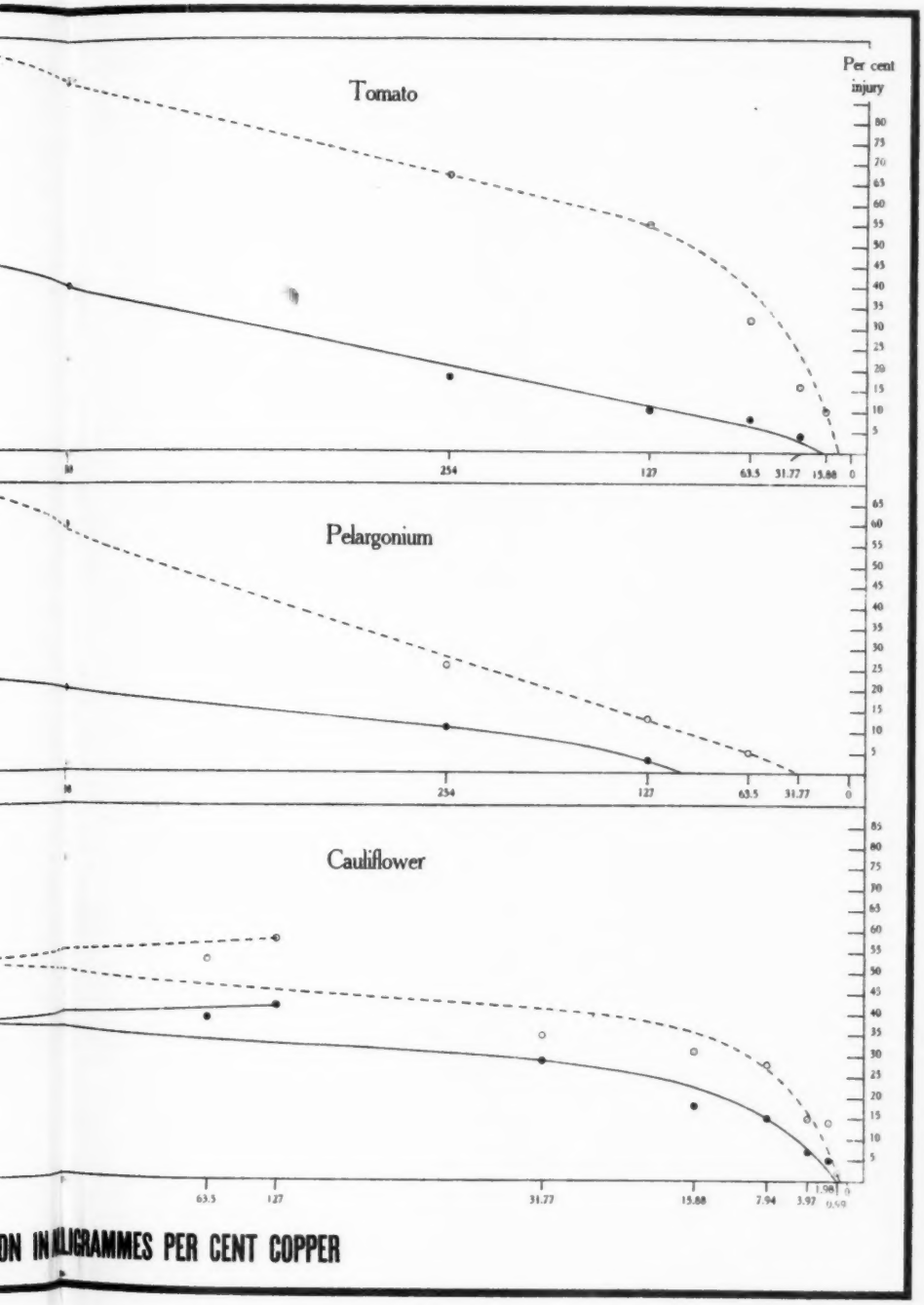

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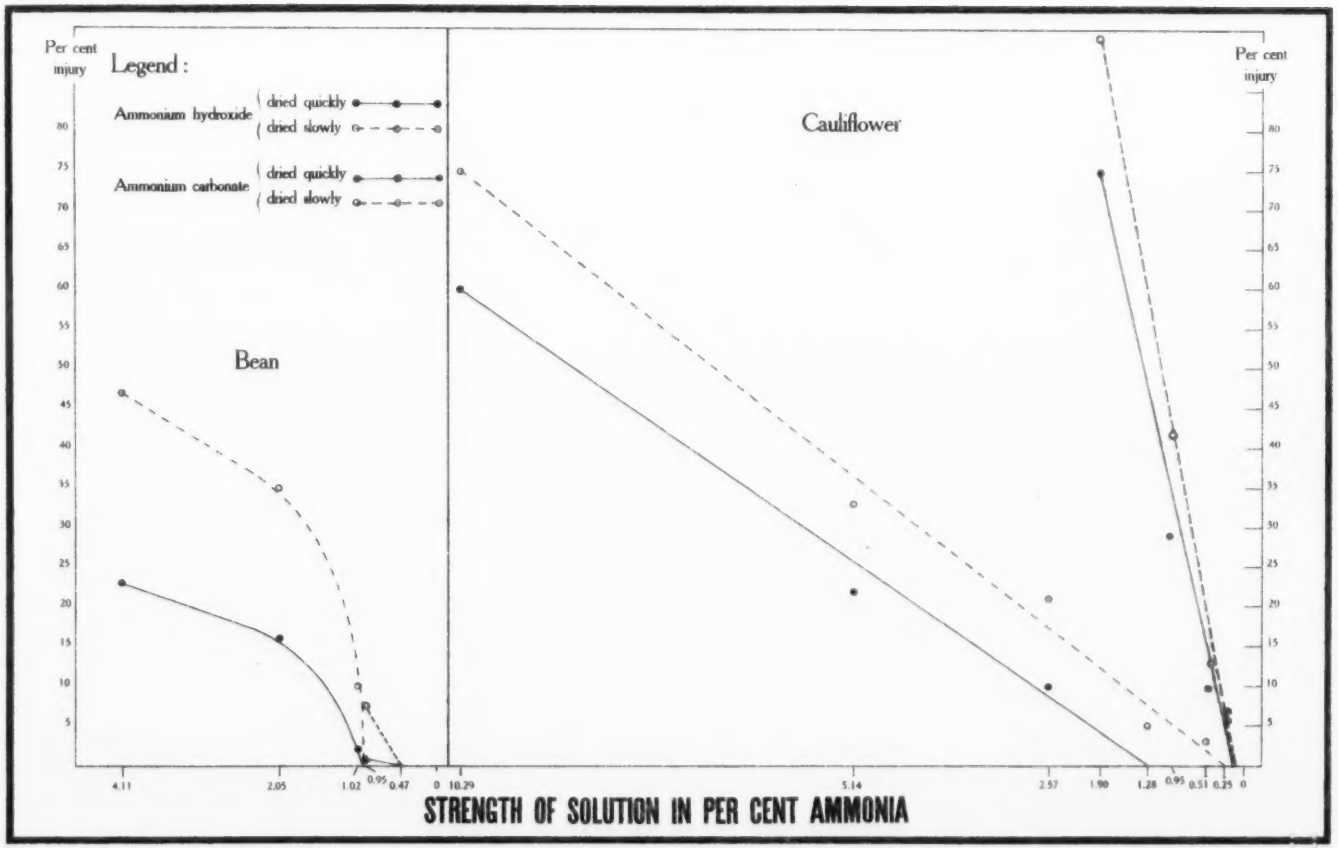

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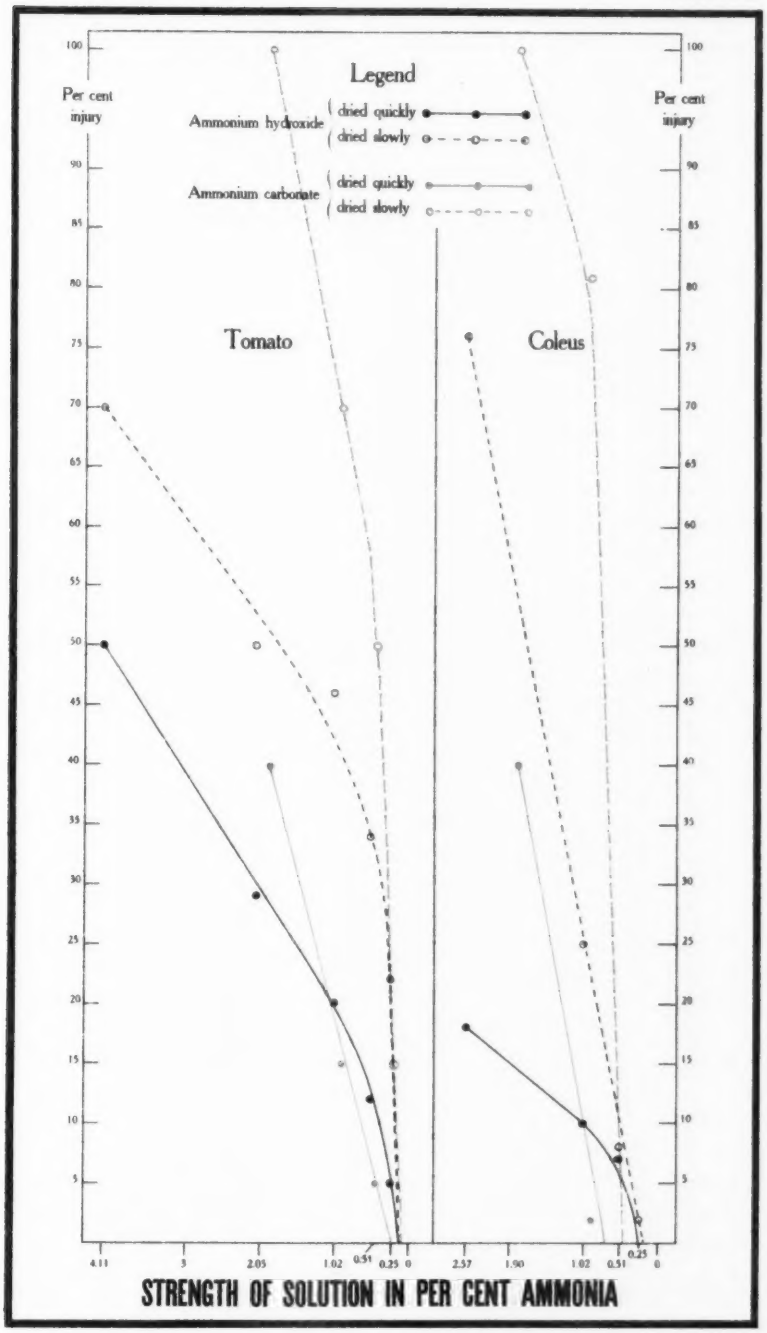

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BACILLUS MORULANS N. SP.

A Bacterial Organism Found Associated with Curly Top of the Sugar Beet

P. A. Boncquet

With Seven Figures in the Text

The writer, in conjunction with Prof. Ralph E. Smith, has been investigating a peculiar and fundamentally important plant disease, the so-called curly top of the sugar beet and related plants. Two joint publications[1] have already been made upon this subject in which there has been briefly announced the discovery of a bacterial organism found constantly associated with the disease in the tissues of affected plants. It has been stated further that, although obtained so regularly and abundantly in cultures

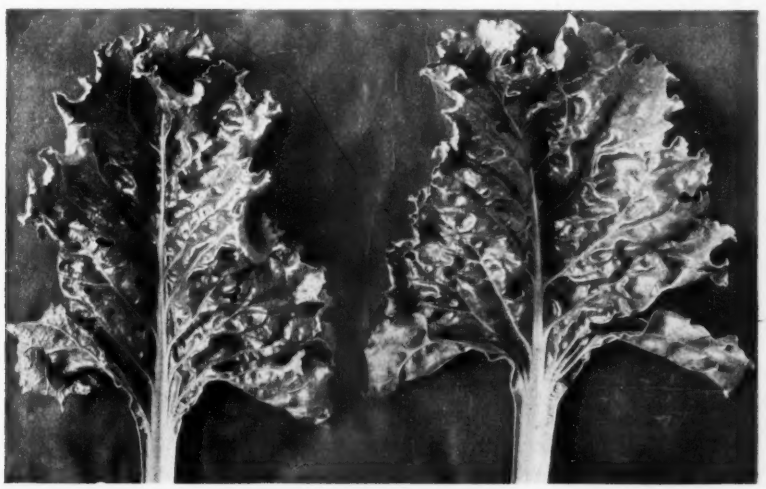

Fig. 1. Wrinkled Type of Beet Leaves

Not curly top, but having in the sieve tubes occasional groups of the bacterial bodies found in great abundance in curly top beets.

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[1] Smith, R. E., and Boncquet, P. A. New light on curly top of the sugar beet. Phytopath. **5**: 103–107. 1915.

——— Connection of a bacterial organism with curly leaf of the sugar beet. Phytopath. **5**: 335–342. 1915.

FIG. 2. SUGAR BEET PLANT TYPICALLY AFFECTED WITH CURLY TOP

from diseased plants or portions of plants, it has not been possible to produce curly top by inoculation with cultures of this organism; that the same organism has been isolated from the surface of beet seed, the surface of normal sugar beet leaves and from the soil about the roots of sugar beets. Also that certain bodies which seem to represent the same organism have been found in great abundance in curly top sugar beets, in the interior of the sieve tubes, accompanying a specific lesion in the phloem, and that similar bodies, in varying but much less abundance, were found in the same tissue in supposedly normal beets or those with various morphological irregularities of the foliage (fig. 1). Whatever may be the entire significance of the organism in question, its peculiarly abundant occurrence in connection with the sugar beet and its apparent relation to curly top have seemed to justify its careful study, and it is the purpose of the present article to describe more in detail the characteristics of this species, to which the name *Bacillus morulans* has been given.

THE DISEASE

The disease of sugar beets called curly top is of annual occurrence throughout the sugar beet growing regions of Colorado, Utah, Idaho and California. The severity of the disease, however, varies greatly from year to year. Some years veritable disasters are produced by curly top, thousands of acres of sugar beets being totally destroyed after all the expense of preparing the ground and planting the crop has been undergone.

Symptoms on leaves

The comparative size of the inner and outer leaves is altered. The inner leaves are dwarfed, the petiole especially becoming shorter and flatter than the normal, while the outer leaves, if already full grown before the disease becomes apparent, maintain their natural size and shape and, for some time at least, their color, although they may finally turn yellow and die prematurely. The first symptom of abnormality plainly visible to the eye is a distinct transparency of the finest venations of the youngest leaves. This transparency starts at the base of the leaf blade. Gradually the abnormality works higher on the leaf until finally the whole leaf is affected. The youngest leaves are first to suffer; the older ones (such as are not already full grown) show the symptoms as their expansion and growth progress. Almost simultaneously with the appearance of the transparency of the veins small warty protuberances appear upon the veins on the under surface of the leaves, eventually even upon those which are of the smallest size visible to the eye (fig. 2). The margins of the affected leaves then begin to curl slightly upward so as to expose the lower

surface. As these symptoms gradually increase, working outward from the innermost to the outer leaves, black spots soon appear in the principal leaf venation and especially in the youngest petioles. These black spots often break out through the cortical parenchyma and epidermis, allowing a black syrupy substance to ooze out, which upon drying produces in severe cases a crusty appearance upon the petioles. The interrupted and irregular expansion of the leaf venation soon brings about a very characteristic leaf curl. This curling for the most part is not parallel with the main midrib but extends more or less along the margin of the leaf and converges at the apex. If the plants are badly affected when very young they may be entirely killed; otherwise when the disease is advanced the outer leaves become yellow and die while the youngest may remain for a long time practically unchanged or occasionally may later resume

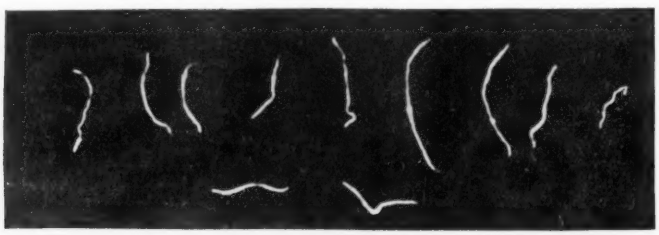

FIG. 3. SUGAR BEET ROOT TIPS, SHOWING CHARACTERISTIC BENDING AND SWELLING

growth and become apparently free from the above-mentioned symptoms. The latter represents a condition of recovery from the disease. In such cases the young leaves are also dwarfed but the venation is normal, the warty protuberances are absent and the leaf does not curl. In this condition, however, the whole leaf is of a denser green color, harder and thicker. The beet seems to have undergone a struggle similar to that which mesophytic plants undergo when they are transplanted to a desert habitat. In such cases the roots may develop to normal size even when the tops are much suppressed.

Symptoms on roots

It is only when the leaves have been thoroughly affected that the roots begin to show abnormality. This is characterized by the multiplication of rootlets. The older ones begin to die while at their base new ones push forth. This continuous destruction of the rootlets brings about a warty appearance, the bases of the masses of rootlets protruding slightly from the surface of the beet. On careful examination it will be observed that each rootlet, after it reaches an appreciable length and before it dies, has

several abnormal bendings; the angle of each bend is slightly swollen and if the rootlet is far advanced the swollen region appears to be necrotic (fig. 3). When the main root is cut transversely the successive rings of vascular tissue appear discolored. On careful examination it will be observed that the phloem is the only part of the vascular system which suffers severely. This phloem discoloration is observed more or less throughout the whole system in the veins as well as in the roots. This, however, only becomes apparent to the naked eye when the disease has reached its severest aspect.

Cause of the disease

It was E. D. Ball[2] who discovered that the sting of the insect *Eutettix tenella* Baker is a necessary factor in the causation of this disease. His observations were confirmed by Shaw,[3] and very fully tested and confirmed by Smith and Boncquet. The latter, however, together with Hartung,[4] proved a fact which had previously been suspected, that *Eutettix tenella* is not the fundamental factor in the causation of this disease, but rather must be a carrier of a second factor, presumably a parasitic micro-organism. This discovery made very important a thorough search for and study of all micro-organisms which possibly could be found in connection with the disease, and it is with this portion of the study of curly leaf that the present article has to do.

BACTERIOLOGICAL INVESTIGATIONS

The methods and detailed results through which the conclusion was reached by the writer that *Bacillus morulans* inhabits constantly and specifically plants affected with curly top, as well as occurring in certain other situations may first be described. It was decided at the outset to make a very thorough and accurate search for any organism which might be present in the tissues of plants affected with the disease. The unfavorable or at best uncertain results reported by previous investigators along this line led to the belief that the problem would be a difficult one,

[2] Ball, E. D. The beet leaf hopper. Utah Agr. Exp. Sta. Ann. Rept. **16:** 16. 1905.

——— The Genus Eutettix. Proc. Davenport Acad. Sci., **12:** 41 and 84. 1907.

——— The leaf hoppers of the sugar beet and their relation to the "curly leaf" conditions. U. S. Dept. Agr. Bur. Ent. Bul. 66, pt. 4. 1909.

[3] Shaw, H. B. The curly top of beets. U. S. Dept. Agr. Bur. Pl. Ind. Bul. 181. 1910.

[4] Boncquet, P. A. and Hartung, W. J. The comparative effect upon sugar beets of Eutettix tenella Baker from wild plants and from curly leaf beets. Phytopath. **5:** 348–349. 1915.

but at the same time a study of the nature of the disease had led to a very strong feeling that some parasitic micro-organism, of which the insect *Eutettix tenella* was presumably a carrier or secondary host, must be involved in this disturbance. Assuming then that the sought-for organism would be an obscure one and difficult to demonstrate by ordinary cultural or histological methods, various special and somewhat elaborate culture methods were attempted.

Preparation of media

The following media which seemed most promising for this purpose were prepared:

Filtered beet juice. For this purpose the plants from which the juice was desired were washed as thoroughly as possible in sterilized water and then ground fine in a meat grinder. In most cases 100 cc. of distilled water was added to each 100 grams of beet pulp and the mass was then covered and left standing for two hours. The crushed material was subsequently put into clean cheesecloth and the juice squeezed out in a press. The juice thus obtained was subsequently diluted twice its volume with n/6 salt solution. Various degrees of dilution have been employed, however, from the original juice up to about ten to one, either in salt solution or water. In some cases the solution was then titrated and brought to the neutral point of phenolphthalein with sodium hydroxid. This juice was now clear, slightly brown and passed easily through a common filter paper. After it had been filtered through paper it was passed through a medium-dense Berkefeld filter candle for purposes of sterilization. An apparatus was especially devised for this purpose, a form of which is described in another article in the present number of Phytopathology. In order to be sure that no contamination had occurred during the manipulation, the tubes after filling were kept in the incubator for two days at 30°C. It is believed that this apparatus and method is worthy of considerable employment in the preparation of culture media for use in plant pathology.

Aseptic, unheated beet slices. These were prepared in the following manner: Sound, healthy beets were selected, thoroughly cleaned and immersed in boiling water for three minutes, in this way sterilizing the surface but not heating or changing in any manner the tissues deeper in the beet. They were then cut into slices with a carefully sterilized knife. Each slice was then put into a sterile petri dish into which previously ordinary nutrient agar had been poured.

Beet broth. Three hundred grams of beet leaves were cut into small pieces and boiled for an hour in 0.5 litre of water. Water was then added to make up to 1 litre and left standing for two hours. It was then filtered through cotton and 500 cc. of this beet extract added to 1 litre of Liebig's broth. The Liebig's broth had previously been prepared in the following way: 2 grams of Liebig's extract, 10 grams of Witte's peptone, and 5 grams of sodium chlorid were added to 1 litre of water. This medium was subsequenty neutralized to phenolphthalein with sodium hydroxid and after addition of the beet juice was brought up to 0.5 per cent acidity with malic acid. The same medium was also prepared with an increased proportion of beet extract.

Artificial media. A protein- and peptone-free medium was composed with the supposition that the organism did not attack the higher nitrogen compounds of the beet. Therefore several of the amino acids were used as the nitrogen supply. Alanin, leucin and tyrosin were used. Asparagin, although not an amino acid was also considered a possible favorable source of nitrogen for the parasitic organism. All these compounds were used in a dilution of 0.5 gram to 1 litre of water. The necessary minerals were added in the following form and proportion:

Magnesium sulfate	0.2 gram
Ammonium phosphate	0.5 gram
Potassium nitrate	0.2 gram
Calcium hydroxid	5 cc. of a saturated solution
Ferric chlorid	trace

These artificial media were sterilized in the Arnold sterilizer for fifteen minutes upon three consecutive days. Special glycerin and glucose media were also prepared. For this purpose 1 per cent glycerin was added to a part of the asparagin medium. So also 5 per cent glucose was added to another portion. The glucose medium was especially used for anaerobic purposes.

Other media. Ordinary media such as nutrient bouillon, potato glucose bouillon, bean pods, milk, litmus whey, nutrient agar and nutrient gelatin were prepared according to the standard methods.

Methods attempted for separating parasites from the plant

In order to separate the assumed parasites from the plant and obtain them in pure culture the following technique was used:

Surface-disinfected plant parts placed in culture medium. The usual method employed in this sort of work consists in soaking the tissue to be employed for a given length of time in mercuric chlorid and then washing off the same with sterilized water before placing the tissue in the culture medium. A need of standardizing this method was felt, inasmuch as there is no assurance, as it is usually described, whether on the one hand the disinfection was sufficiently thorough to kill all surface organisms or whether on the other hand the material was washed sufficiently to remove all the mercury and prevent its being carried over into the culture medium. The method consists in dry sterilizing a number of cotton-plugged flasks of 50 cc. capacity or any other desired size. At the same time larger flasks, likewise cotton-plugged and filled with distilled water, are made sterile in the autoclave. Other requisites are supplies of 95 per cent alcohol and 1 to 1000 solution of mercuric chlorid in water. The material from which cultures are desired, after thorough wiping with cotton swabs in 95 per cent or absolute alcohol in a photographic tray, is cut into convenient sized fragments, but no smaller than necessary. These are placed in one of the empty sterilized flasks and covered for a moment with the alcohol for the purpose of removing air bubbles. The alcohol is immediately poured off again and the flask nearly filled with mercury

solution so that all the material will be submerged. This is allowed to remain for the desired length of time, depending upon the nature of the tissue. The petioles and main veins of sugar beet leaves, especially fairly old leaves, will usually stand twenty minutes, but with leaf blades and other more delicate material ten minutes has been found the maximum time which can be used without too severe burning. Cut surfaces will naturally absorb more of the solution than those protected by the natural covering of the plant, and this can be taken into account both in consideration of the length of time which the tissue will stand without being burned by the mercury and also the time necessary for washing it out again. On this account it is best to cut the tissues as little as possible before disinfecting. After the desired time has elapsed a piece of brass wire gauze, bent to form a cap over the mouth of the flask, is sterilized in the flame, placed in position and the mercury poured off. The flask is then filled with sterilized water from the large flask and the water of this first washing, after having the material well shaken up in it, is poured off immediately and more water poured in. The process of pouring off and refilling is then continued at gradually increased intervals; the length of each must depend upon the nature of the material. It was found, however, that if the amount of material in the flask is comparatively small in proportion to its capacity, which should always be the case, six changes of water, extending over a period of two hours, is amply sufficient in every case. In this case the first five changes can be made during the first hour and the last one at the end of the second hour. If one wishes to practice extreme caution the mouth of the flask may be flamed and the cotton stopper replaced after each change of water, but this has not been found necessary so long as the amount of water is sufficient to thoroughly submerge all the material. In our work the wire screen is usually left over the mouth of the flask and this is freshly flamed before each change of water. After the process is completed the material is taken out of the last water with flamed forceps, broken into small pieces if necessary and thoroughly crushed with the same instruments and dropped into the culture liquid.

Piece cultures. It was thought that a gradual adaptation from the plant in which the organism is living to the medium in which it was attempted to grow it might be necessary to insure success; therefore the diseased tissue was so transferred as to disturb as little as possible the cells of the beet. For this purpose glass tubes were drawn out to 2 mm. diameter. After sterilizing by heat they were aseptically inserted into the diseased regions of the beet to a depth of 1 cm. The tube was then withdrawn, bringing with it a portion of the beet tissue and the terminal part containing the tissue was carefully broken off with sterilized forceps

and dropped into the medium. In this way both ends of the tissue slightly protruded from the glass tube and came into direct contact with the culture medium. The slow diffusion of the latter was supposed to secure a gradual change of habitat in such a way as not to hinder too severely the growth of the parasite. Tissue was thus removed from the petiole, from inside the root and from the larger veins of the leaf, after surface sterilization with a flame or boiling water, afterwards cutting into the interior with a sterilized knife and then introducing the glass tube to take out a small core of tissue.

Results of isolation experiments

The various special methods described were carried out very carefully. The result was that in almost every instance cultures from curly top tissue in all the various media described, and especially those which contained glucose, showed a heavy growth after twelve hours of incubation at 20°C. This result was practically uniform wherever diseased material had been used. Occasionally growth also appeared in cultures from supposedly normal plants, but in by far the great majority of cases such cultures remained sterile. This seemed to indicate that the organism was not peculiarly difficult to isolate, judging from its abundant growth on such a wide variety of media. Nevertheless a painstaking work was undertaken in order to complete the thorough study which had been planned. The anaerobic cultures also proved to be invaded by the same organism. Here, however, the growth was less abundant and extremely slow. Several days elapsed before any colonies were visible. A great deal of effort was further spent on work with all kinds of media but always the same organism grew abundantly. Contaminations naturally occurred now and then but the fact was most decidedly apparent that the one species announced by Smith and Boncquet predominated in the tissues of curly top plants to the practical exclusion of all others. The special culture methods described above are given in some detail, inasmuch as they may contain suggestions of value in similar work. Having found that this organism grew so easily and abundantly upon ordinary media, the use of special preparations was abandoned in the attempts to isolate the organism from plant tissues and the work was carried on entirely with standard bouillon to which 5 per cent glucose had been added. The object of the glucose was to promote the growth of the characteristic zoogloeae of this organism, rendering its identification in the original tubes easy without plating. By occasional plating, as a check on the work, it was soon possible to identify this organism very accurately by microscopic examination of tubes which showed the characteristic ring formation at the surface of the liquid. The

TABLE 1

Results of bacterial isolation experiments by cultural methods

(Tubes incubated at 28°C. *Disease* means curly top)

MATERIAL	NUMBER OF TUBES	RESULT
Petioles of curly top beets	6	5 tubes developed *B. morulans* within two days
Petioles of normal beets	6	Tubes remained clear for a week, when they were discarded
Petioles of diseased beets	9	8 tubes developed *morulans*
A leaf showing curly top symptoms on half of blade and in corresponding half of petiole. Other side appeared normal. This material from affected half of petiole with black streaks	5	All developed *morulans*
Corresponding half of blade	3	2 tubes developed *morulans*
Normal-appearing half of petiole	6	5 tubes developed *morulans* two days later than those from blackened part
Normal-appearing side of blade	3	No development
Petiole of a diseased center leaf	4	3 with *morulans*, 1 doubtful
Petioles of good-sized leaves from 5 different normal-appearing beets	10	2 tubes from 1 leaf both with *morulans;* others all clear
Typically diseased leaf with very slight dark streaks in the petiole	6	All developed *morulans*
Badly affected petiole of same beet. Pieces cut out with flamed scalpel	4	All developed *morulans*
Petioles of 4 beets from insect-proof cage. No sign of disease	8	Tubes from 2 plants remained clear; those from other 2 became slightly cloudy after several days, but no *morulans*
Petioles of 2 slightly diseased leaves. Pieces cut out with flamed scalpel	13	All tubes apparently containing pure cultures of *morulans*
A yellowish aster leaf	2	Remained clear
An old yellowish beet leaf without curly top. Tissue still sound	4	Some fungous growth. No *morulans*
Leaves of a somewhat abnormal-appearing beet but not with curly top	6	Some growth, but no *morulans*
Healthy-appearing leaves of aster, chrysanthemum, dahlia, tomato, bean, lettuce and raddish	24	11 tubes with fungi and bacteria, remainder clear. No *morulans* found
Petiole of typically diseased leaf, no disinfection	6	Very abundant growth of *morulans* intermixed with other organisms

TABLE 1—*Continued*

MATERIAL	NUMBER OF TUBES	RESULT
Petiole of slightly diseased leaf	2	Both very abundant *morulans*
Blade of same between veins	2	Both remained clear
Typically diseased leaf; scraped out interior portions of petiole with flamed scalpel after cleaning off epidermis	4	3 tubes developed *morulans*. 1 doubtful
A young leaf visibly affected on one side and very slightly at the base of the other side. Tissue taken from the most diseased side at base	4	All developed *morulans*
Terminal portion of diseased side, less visibly affected	4	2 developed *morulans;* 2 clear
Slightly affected base of other side of same leaf	4	1 developed *morulans;* 3 clear
Not visibly affected terminal portion of last	4	All remained clear

NOTE.—The last four are from the leaf illustrated in Phytopathology **5**: 106. The most elaborate precautions were taken to secure perfect surface disinfection and avoid contamination. These tubes in which growth appeared were plated out and found to contain pure cultures of *morulans*. The leaf was perfectly sound, showing only a slight roughening of the veins on the affected portion.

appearance to the eye of this ring, supplemented by microscopic examination, finding it to be composed of the characteristic zoogloeae, supplemented by occasional plating, is amply sufficient to identify this organism. A number of typical examples of isolation experiments with sugar beets are shown in table 1.

Several hundred illustrations similar to those shown in table 1 might be given. The results varied somewhat with the perfection of technique

TABLE 2

Bacillus morulans upon sugar beet seed

MATERIAL	NUMBER OF TUBES	RESULT
Beet seed imported from Germany, 1 dropped into each bouillon tube, with no previous treatment	10	At least 7 developed an abundance of *morulans*, mixed with other organisms
Similar seed previously soaked for twenty minutes in mercuric chlorid and washed in sterilized water	10	All clear

and in individual cases, but a mass of evidence was collected to indicate that this organism exists regularly in the interior of the foliage of sugar beets where the visible symptoms of curly top occur and that it does not develop in cultures from normal foliage or even the normal-appearing portions of partially affected leaves; also that it does not occur in the interior of beet leaves which may be yellow or sickly from ordinary causes.

Cultures from seed. Many attempts similar to those shown in table 2 were made to isolate the organism from sugar beet seed. The uniform result was that almost every unsterilized beet seed dropped into a tube of bouillon developed a very luxuriant growth of *Bacillus morulans.*

TABLE 3

Bacillus morulans from soil

MATERIAL	NUMBER OF TUBES	RESULT
Pinches of soil from about the roots of a diseased beet	4	*Morulans* was abundant in several of the tubes
Pinches of soil from about the roots of a normal beet in insect-proof cage	4	Some *morulans* present in the mixed growth resulting

Cultures from soil. That the organism is present in some soils is indicated by the data presented in table 3. The work was rather crude but certainly *B. morulans* was abundant in the soils examined.

Cultures from unsterilized foliage. Cultures made from unsterilized leaves of the sugar beet (table 4) show that the organism is common as a saprophyte upon the leaves of the plant, but in all cases when leaves similar to these were thoroughly disinfected no growth was obtained.

TABLE 4

Cultures from unsterilized foliage

MATERIAL	NUMBER OF TUBES	RESULT
Leaves of normal beets	10	Several contained an abundance of *morulans*

Unsterilized leaves of many other plants were also tried but the resulting growth was so mixed that no safe conclusions could be drawn. The only certain developments of *morulans* occurred in tubes inoculated with pieces of chrysanthemum leaves.

Cultures from sugar beet leaves with types of disease other than curly top. The fact that bodies resembling bacteria have been seen with the microscope in sugar beet leaves not affected with curly top, but affected with

various types of morphological abnormality, has been referred to in our recent article in Phytopathology. These various foliage types have never been definitely classified or described nor has the extent of the occurrence of this organism in other tissues been learned. In this cultural study,

FIG. 4. MOTTLED LEAF OF SUGAR BEET

however, two at least of these forms gave the organism in abundance (table 5). In one of these, which we shall call mottled leaf (fig. 4), the blades of the leaves are strongly mottled with green and pale areas in a very characteristic manner. In the other type which we call black edge or black tip (fig. 5), the green leaves begin to die and turn decidedly black

about the margin or at the tips, with a yellow band of tissue between the black and the normal green. In some such leaves the development is very much suppressed, even to a point where the leaf consists of scarcely more than a petiole with a small blackened tip.

FIG. 5. BLACK EDGE AND BLACK TIP OF SUGAR BEET LEAVES
Black tip (left) and black edge of sugar beet leaves

Relation of mottled leaf and black edge to curly top. For some time it was believed that these conditions represented types or stages of curly top. Later, however, it was found that they occurred under circumstances which made this seem practically impossible. Further, when the experiment was tried of placing *Eutettix tenella* upon such plants the typical disease developed promptly.

TABLE 5

Cultures from sugar beet leaves with types of disease other than curly top

MATERIAL	NUMBER OF TUBES	RESULT
Yellowish area of young "black edge" leaf taken from between black and green portions. Thoroughly disinfected	2	Both gave a strong growth of *morulans*
Similar to last, but not disinfected	4	Very vigorous and apparently nearly pure growth of *morulans*
Similar material, disinfected	4	All with *morulans*
Similar material, disinfected	4	All with *morulans*
Typical "mottled leaf;" not disinfected. Blade, petiole and veins	9	All produced *morulans* in abundance
Petiole of "black edge" leaf, disinfected	4	All appear to have pure cultures of *morulans*
Petioles of normal appearing leaves from healthy plant. Very carefully disinfected	5	All tubes clear
Petiole of decidedly "mottled leaf." Inner tissue removed with flamed scalpel	4	Very vigorous growth of *morulans*

Further study of the organism, which was uniformly present in diseased beets

Although the organism was able to grow most abundantly on the common culture media, the peculiarity of this growth under all circumstances was of such a nature that for some time a continual contamination was suspected. Each separate colony seemed always to have two kinds of bacteria, very distinct in form. Very active bacteria were always observed at the edge of the colonies, while capsultated bacilli were generally observed in the middle. Therefore, before any further study of the organism was taken up, repeated efforts were made to separate these two widely distinct forms. For this purpose, the calcium carbonate and the India ink method for separating the individual organisms previous to plating them were resorted to.

Calcium carbonate. To 10 grams of calcium carbonate enough water was added to form a milky paste. This was subsequently introduced into a 200 cc. Erlenmeyer flask and sterilized in the autoclave. After the necessary cooling several young colonies of the bacteria were introduced into the semi-liquid mass and shaken for two hours so as to separate each individual organism from the other. From this paste, several plates were poured in the usual manner. They were incubated at 37°C. and closely examined as soon as any sign of development occurred.

India ink method. For this purpose special Chinese ink, prepared by Grübler, (*Punkttusche*) was used. A 15 per cent nutrient gelatin was made and poured into clay-covered petri dishes. Special care was given to prevent condensation water from flooding the medium. The Grübler's ink was diluted twenty times with n/6 glucose solution and sterilized in the autoclave. In a sterilized, empty petri dish ten drops of the ink were put in a row. The first drop was inoculated with a small amount of bacteria from a twelve-hours-old streak culture. The bacteria were thoroughly mixed with the ink of the first drop. Then a loop of this was transferred to the second drop and also thoroughly mixed. This transfer was repeated in the same way with the remaining drops in the dish. From the tenth drop, with a sterilized drawing pen, a small amount was taken. Small dots were made with the pen on a gelatin plate in such a way that the surface pellicule of gelatin remained uninjured. These ink dots were left to dry for two minutes then covered with a sterilized cover-glass. A small drop of immersion oil was subsequently applied to the cover-glass and the whole petri dish was brought to the microscope for examination. Each black point was then examined with microscope until one was found which contained one single organism. The organism appeared as a translucent dot on a black field. Its development was closely followed; the first division was distinctly noticed after half an hour; it multiplied rapidly; all the individuals were motile; they liquefied the gelatin slightly and moved about very briskly in the liquid under the cover-glass. After six hours some of the organisms became sluggish and gradually lost their motion. They increased in size and formed a capsule. Repeatedly they divided in the same capsule, stretching the jelly-like membrane more and more. The newly formed organisms within the original capsule also encapsulated in their turn (figs. 6 and 7). At the same time the individuals on the rim of the colony multiplied and remained motile. The double form of the bacillus was in this way clearly explained and proved.

IDENTITY OF THE ORGANISM FOUND IN CURLY TOP BEETS

A study of the literature of the subject shows that the greatest similarity to our organism of any described species is presented by that described by Arthur and Bolley[5] as *Bacterium Dianthi* as the cause of a leaf spot of the carnation. In its morphology, so far as described by these writers, this organism is very similar to ours, the resemblance being made pronounced by the development of characteristic zoogloeae. In biological behavior, however, the two organisms cannot be accurately compared, since the

[5] Arthur, J. C. and Bolley, H. L. Bacteriosis of carnations. Indiana Agr. Exp. Sta. Bul. 59. 1896.

work of Arthur and Bolley was carried on at a time when bacteriological technique was not standardized upon modern lines. One noticeable difference exists in respect to growth upon an acid medium, *B. Dianthi* being said to grow best under such circumstances, which is not the case with our organism. The description of the bacterial organism given by Arthur and Golden[6] and again by Miss Cunningham[7] as the cause of the so-called Indiana sugar beet disease, is similarly subject to uncertainty, but if this work was accurately done the organism must certainly have been different from ours in that it is said to be a particularly active gas former, which feature is totally lacking in our organism. It seems proper to mention here, however, the fact that Professor Arthur in a recent personal letter states that the accuracy of all this early work performed under his direction is open to some doubt on account of the undeveloped condition of bacteriological technique at the time and he expresses the opinion that the organisms found by Bolley, Miss Golden and Miss Cunningham were very likely identical.

The organism described in the unpublished work of Schneider[8] as *Bacillus californiensis*, which was isolated from curly top beets in California, seems again in its morphological characteristics to be entirely similar to ours and we feel little doubt that Schneider and the present writer had the same organism before them. Schneider found his organism also very abundant in sugar beet soils and upon the surface of the plants. He attributes a stimulative effect to this species, when applied in pure culture to sterilized beet seed or to the foliage of young plants.

The organism described by Düggeli[9] as being abundantly and often exclusively present upon the surface of various plants and seeds, seems also very similar to ours in form and size, formation of zoogloeae, color, saprophytic habitat and most biological characters. This was named by Düggeli *Bacterium herbicola aureum*, but "said to be the same as the *Bacillus mesentericus aureus*, isolated by Winkler from the surface of plum leaves." The latter statement confuses the identification.

DESCRIPTION OF THE ORGANISM

Summing up the whole situation, we feel justified in describing our organism as a new species on account of the incomplete and doubtfully

[6] Arthur, J. C. and Golden, K. E. Disease of the sugar beet root. Indiana Agr. Exp. Sta. Bul. 39, pt. 3: 54. 1892.

[7] Cunningham, C. A. A bacterial disease of the sugar beet. Bot. Gaz. **28:** 177–192. 1899.

[8] Schneider, A. The California beet blight. Spreckels Sugar Co. Exp. Sta. Rept. **23:** —. 1906. (Unpublished.)

[9] Centbl. Bakt. II, **12:** 602 and 695; **13:** 56 and 198. 1904.

accurate descriptions of those species which more or less resemble it, and the fact that none of them corresponds throughout.

Bacillus morulans n. sp.

Morphology

Vegetative cells. Grown in Liebig bouillon for sixteen hours at about 20°C., oval to short rods, single or in pairs. Grown at 37°, short rods in pairs or in short chains.

Size. Length 1.5 μ; breadth 0.9 μ; extreme length from 1.5 to 2 μ.

Capsules. Easily observed in 1/1000 glycerin bouillon after twenty-four hours and also in milk media (figs. 6 and 7).

Motility. Very active on agar and in bouillon, when grown at 37° for twelve hours.

Flagella. Stained by Zettnow's method; four long peritrichial flagella (figs. 6 and 7).

Pleomorphism. Cocciforms observed in glucose bouillon tubes and blood serum media after thirty days.

Stain. Easily with watery fuchsin, decolorized by Gram's method.

Cultural features

Gelatin plate neutral to phenolphthalein. Form, round to irregular; surface elevation, flat to convex contoured; internal structure, refraction strong, hyaline, moruloid; zoogloeae very marked; edges, entire to undulate; optical characteristics transparent to butyrous; consistency, viscous. Each colony is surrounded with many secondary colonies, appearing as small, oily drops of high refractive power. The appearance of zoogloeae is very noticeable in the middle of the colonies.

Gelatin plates 1.5 acid to phenolphthalein. The entire mass is a zoogloea, lobed and irregular in outline; the colony is slightly colored; orange-yellow, no surrounding colonies noticed.

Gelatin streak. After five days: growth, linear; margin, continuous; surface relief, flat to convex; light transmission, butyrous; color, yellow-orange; luster, glistening; consistency, viscous. The water of condensation has a yellow sediment.

Gelatin stab. After twenty-two hours, top growth: size, 5 mm., irregular contoured; pulvinate to capitate, light orange in color; viscous in consistency, luster shining.

Gelatin streak. Filiform to slightly beaded. After fifteen days, liquefaction of medium; crateriform with a yellow sediment. After twenty days, liquefaction stratiform; yellow pigment, decreased by absence of oxygen.

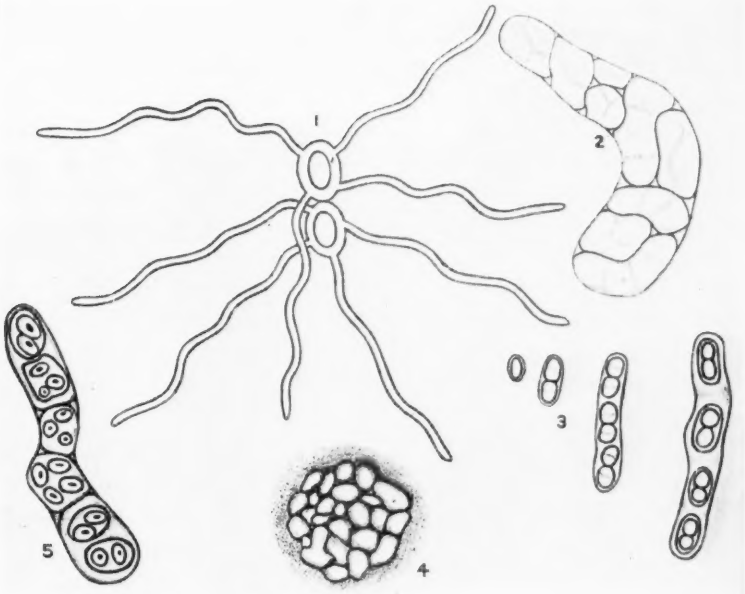

FIG. 6. MORPHOLOGY OF BACILLUS MORULANS

1, A chain of two individuals, showing flagella.

2, *3*, *5*, development of capsule and zoogloea from one individual. *4*, mass of zoogloeae.

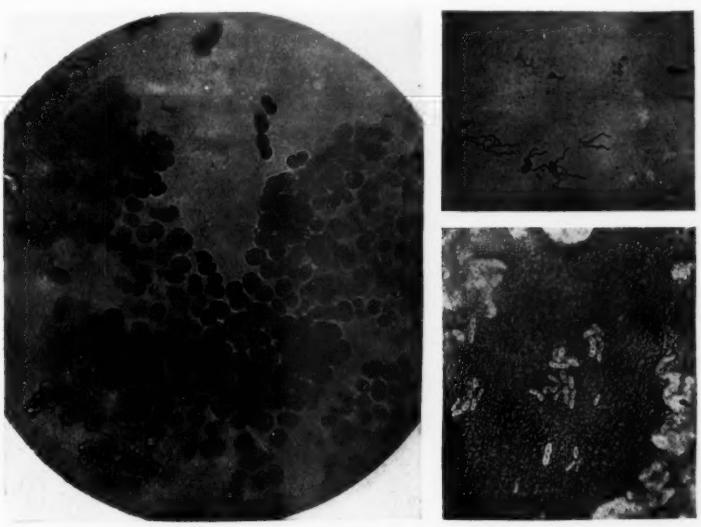

FIG. 7. BACILLUS MORULANS, SHOWING CAPSULES AND FLAGELLA

Agar. The colonies are extremely variable according to the density of growth, the moisture and the temperature.

Milk. Peptonisation of casein in fifteen days at 37°. The reaction is alkaline to Azolitmin.

Litmus whey. Remains clear, alkaline reaction.

Bouillon tubes. Opacity begins after eight hours at 37°, a pellicule forms in twenty-four hours or less. The color of the pellicule and the ring is dull, soft gray; thick, viscous and consists of conglomerate zoogloeae. These are generally oval, but may be linear and all united in chains.

Deposit, forms after two days incubation at 37°. Deposit is in the beginning slight, and finally yellow. The amount of deposit and the intensity of the color increases, however, rapidly. After ten days, a decidedly deep yellow-orange has developed. The deposit is compact and viscid on agitation.

Potato streak. After twenty four hours at 37°. Size, 2 mm.; sharp, linear; margin, continuous; color, yellow, homochromous; luster, glistening; texture, homogeneous. No liquefaction of potato and no gas formation.

Physical and biochemical features

Reaction. In carbohydrate-free media the reaction is alkaline; in carbohydrate media, the reaction is acid, except in lactose, where the reaction is slightly alkaline. See table 6.

Nitrate Liebig broth. After twenty-four hours at 37°, strongly reduced to nitrite.

Indol. Not produced in peptone solution after ten days.

Optimum temperature. 37°, measured by the amount of acid produced in 1 per cent glucose after five days. Acidity was 2.5.

Thermal deathpoint. Six-hours culture in bouillon; 54°C. in ten minutes.

Carbohydrate fermentation. Shown in table 6.

Resistance to mercuric chlorid. Six-hours culture on bouillon agar streak killed in 1/25,000 to 1/30,000 in ten minutes.

Relative growth in acid and alkalin media. Determined by the appearance of cloudiness in the tubes. Grows best on neutral or slightly alkalin media. Five per cent in acid apparently stops all growth; 7 per cent in alkaline: same.

Gas production. No gas is produced. See table 6.

Relation to free oxygen. Aerobic; facultative anaerobic.

TABLE 6

Carbohydrate fermentation of Bacillus morulans

(Incubation: 37°C., medium neutral to azolitmin)

MEDIUM: 1 PER CENT PEPTONE AND 1 PER CENT CARBOHYDRATE	AFTER 1 DAY	AFTER 2 DAYS	AFTER 3 DAYS	AFTER 4 DAYS	AFTER 5 DAYS	ACIDITY AFTER 5 DAYS	GAS AFTER 5 DAYS	DEPOSIT	FILM	RING	REACTION	
											Closed arm	Open arm
						$\times 10^{-5}$						
Dextrin	0	0	AB*	AB*	AB*		0	p	a	a	acid	alk.
Inulin	0	0	0	0	0	0	0	a	a	x	0	0
Amygdalin	0	0	0	0	0	0	0	a	a	a	0	0
Salicin	A	A	A	A	A	1.8	0	p	a	a	acid	acid
Glycerin	0	0	0	A	A	0.1	0	a	a	b	acid	acid
Lactose	0	0	B	B	B	0.2	0	a	a	a	basic	basic
Laevulose	A	A	A	A	A	2.3	0	b	b	p	acid	acid
Galactose	A	A	A	A	A	2.1	0	b	b	p	acid	acid
Dextrose	A	A	A	A	A	2.5	0	b	b	b	acid	acid
Saccharose	A	A	A	A	A	1.6	0	p	b	p	acid	acid
Mannit	A	A	A	A	A	1.3	0	p	a	x	acid	acid
Maltose	A	A	A	A	A	1.3	0	p	a	p	acid	acid
Rhamnose	A	A	A	A	A	1.3	0	p	a	x	acid	acid

NOTE.—*A*, acid; *B*, basic; *p*, permanent; *b*, abundantly present; *a*, absent; *x*, more or less present; *, acid on top and basic in tube.

Pathogenesis

One loop from a twelve-hours-old streak culture on bouillon agar introduced intravenously in a rabbit, caused death within twenty-four hours.

On *Dianthus incarnata*. The young unfolded leaves, when unrolled and covered with an abundant suspension of bacteria, developed small necrotic regions. The necrotic regions are watery and translucent on the edges, slightly elongate or irregular in outline, following the venation. The inside of the necrotic regions is slightly brown.

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A NEW APPARATUS FOR ASEPTIC ULTRAFILTRATION

RALPH E. SMITH

WITH TWO FIGURES IN THE TEXT

In various investigations of so-called nonparasitic or physiological plant diseases, as well as in numerous animal diseases in which the presence of an ultramicroscopic organism is suspected, the juices or body fluids of affected plants or animals have often been subjected to filtration through Berkefeld, Chamberland and similar filters in order to remove bacteria and other organisms of microscopically visible size. While inoculations with such filtered juice have been made frequently, and in some cases (tobacco mosaic) with positive results, the possibility of demonstrating the presence of a parasite in the filtered juice by its possible growth when all other organisms are excluded, seems to have received little attention so far as plant diseases are concerned.

Another object in producing an aseptically filtered juice is that of obtaining a sterile, unheated plant extract as a culture medium, as mentioned by Mr. Boncquet in another article in this number of Phytopathology. In either case the juice may be varied in concentration or reaction, any desired substance which will pass through the filter may be added to it, and in many ways use may be found in the investigation of plant disease for aseptically filtered and preserved or cold-sterilized juices. Various devices for this purpose have been described but all of them, so far as the writer is aware, are clumsy and of very doubtful efficiency. Those in which the candle and the liquid to be filtered are in a tube directly above the receptacle or outlet for the filtered juice are especially undesirable, since the slighest leakage may result in contaminating the filtrate with the unfiltered juice and thus defeating the whole object of filtration. Rubber stoppers are always open to suspicion. Arrangements in which the filtrate is caught in an open or a cotton-stopped vessel and then poured into the culture tubes in the open air are certainly far from safe. Those in which the liquid is forced through the candle from the inside outward are objectionable on account of the quick coating over of the inner surface with sediment, which, on the outside, may be mostly brushed off. In the apparatus illustrated here the only possibility of contamination is from the air and cannot occur from the unfiltered juice. In other words, it is absolutely certain that nothing

which is in the original juice can get into the filtrate except by passing through the pores of the candle.

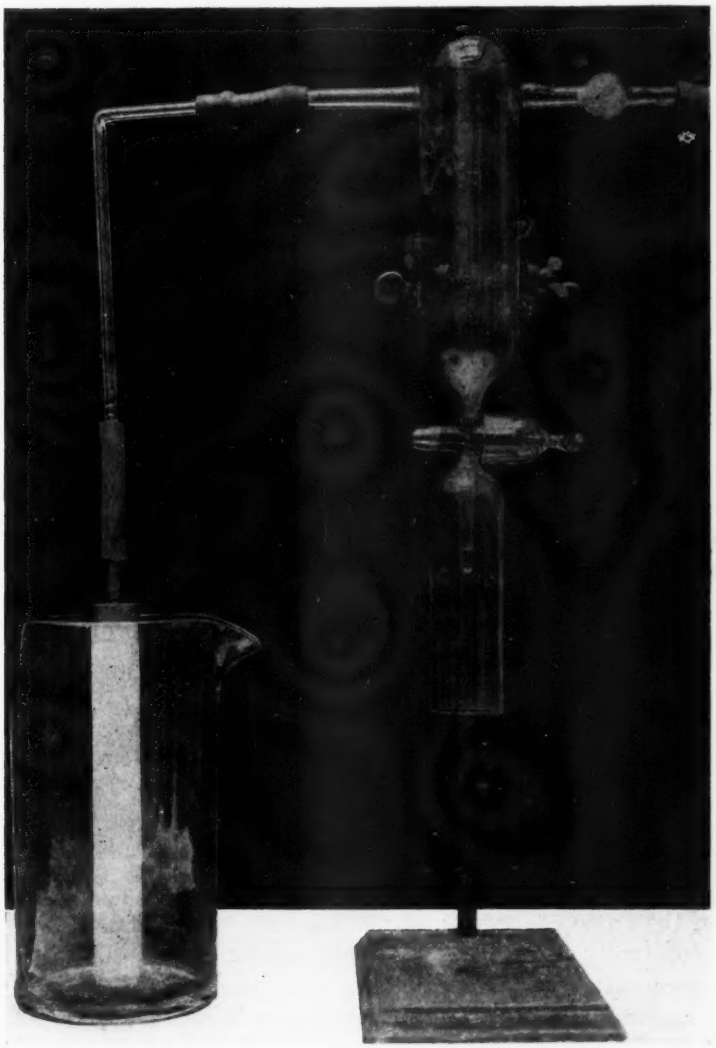

FIG. 1. FILTER APPARATUS, BEFORE WRAPPING

In this apparatus the liquid is drawn up through the filter into the large upper tube and run off below by means of the stop-cock and outlet into sterilized test-tubes. Before commencing operations the two joints

in the line of tubing from the candle to the upper reservoir are wrapped closely with cotton, extending beyond the ends of the rubber connections and for about an inch down over the candle itself. Over these are put again still longer cotton wrappings and finally a layer is put on covering the whole line completely from a point two inches below the upper end of the candle up to the reservoir. The whole region around the stop-cock is similarly wrapped with several layers of cotton, with a large plug of the same material in the lower opening and a wrapping over this and up around the tube. It is the intention that all the joints shall be air-tight and in this apparatus the number of joints and chances of contamination are less than in most similar devices. (Compare for example the arrangement figured on page 65 of Marshall's Microbiology.) The cotton wrappings are added, however, as an extra precaution.

After the apparatus has been put together and wrapped it is sterilized in the autoclave. At the same time a supply of cotton-plugged test-tubes is sterilized by dry heat. The apparatus is then set up on a table convenient to a gas burner and some of the juice to be filtered poured into the beaker, taking care not to allow the cotton at the upper end of the candle to touch the liquid. The water vacuum pump is started, using as little suction as possible, and allowed to operate until a supply of the filtered juice has been collected in the reservoir. If the surface of the candle becomes too much clogged it may be cleaned with a soft brush, but it is desirable before commencing the filtration to remove as much solid material from the juice as possible by filtering through cloth, paper and sand. As soon as enough juice has been filtered the cotton plug closing the bottom of the main tube is removed; one of the sterilized test-tubes is held in one hand, the plug removed and discarded, the tube thoroughly flamed several inches down from the top and then passed up into the lower end of the apparatus below the outlet tube. With the other hand the stop-cock is turned and some of the liquid run into the tube. After shutting off the cock, another tube is taken from the basket with that hand, while the tube just filled is withdrawn and its open end held in the flame. The plug of no. 2 is now transferred to no. 1, both tubes constantly flamed, no. 1 is laid aside and no. 2 filled, continuing the process with as many tubes as desired.

The apparatus figured is 14 inches long over all, and holds 200 cc. of liquid. Different sizes may of course be made and it is very probable that some of those interested in the matter may be able to suggest improvements upon the apparatus. A continuous glass tube might be used between the reservoir and filter candle, thus eliminating one joint, but it has been thought that the increased liability of breakage would more than offset any disadvantage. It might also be possible to cement

the joints in this tubing with some air-tight material, and thus eliminate any possibility of leakage at those points. This would leave the stopcock as the only possible place where anything could get in without passing through the candle. Its efficiency depends upon the tightness with which it fits together and upon its being well greased. Any leakage at this point may be easily detected by the bubbles of air passing up through the liquid.

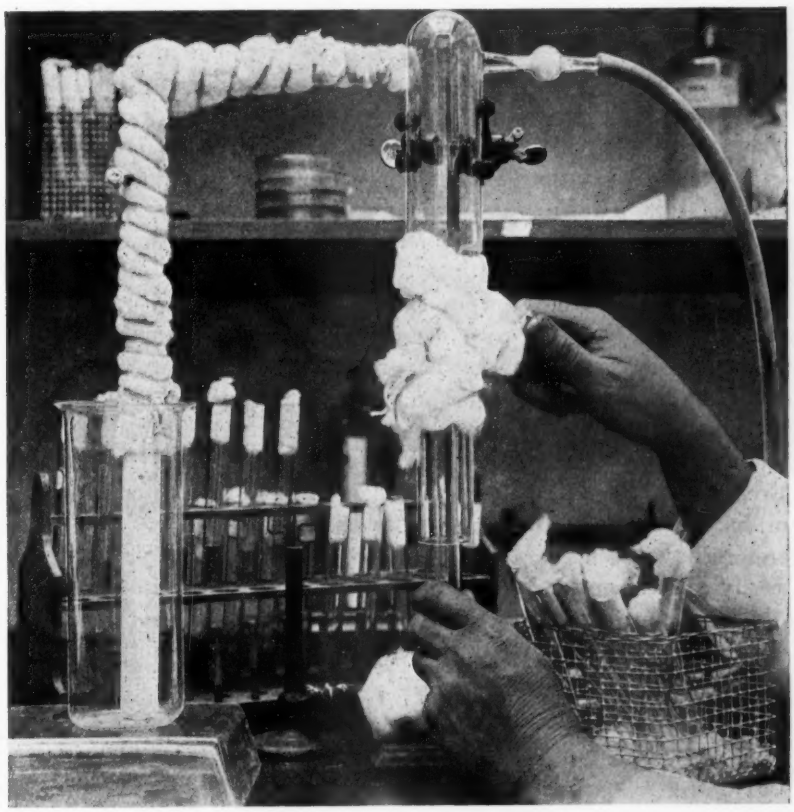

FIG. 2. METHOD OF USING FILTER APPARATUS

The apparatus shown in the illustration was constructed by the Braun-Knecht-Heimann Company, of San Francisco, after sketches by the writer.

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FACTORS AFFECTING THE PARASITISM OF USTILAGO ZEÆ

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Because certain facts seemed to indicate a different life history from that usually credited to *Ustilago Zeæ* (Beckm.) Unger., on corn, field inoculation experiments were carried on during the summers of 1913 and 1914. The results of the experiments were such as to make a more detailed investigation of the spores and sporidia highly desirable.

The works of Von Waldeheim (10), Brefeld (2, 3), Hitchcock and Norton (6), and Clinton (4) have given us the salient points in the life history of this parasite, and numerous other investigators have made additional contributions from time to time, showing that the control of the fungus is a difficult problem. The spores of the smut are widely distributed by the wind and are produced in large numbers throughout the growing season. They are capable of germinating immediately and, in a suitable medium, they produce immense numbers of sporidia which may bud in a yeast-like manner and produce a host of others. The production of the sporidia in large numbers in the field is possible in such places as manure or compost heaps.

Brefeld (2) conducted a few experiments from which he concluded that sporidia are short lived, dying in five weeks when dry. The sporidia therefore have been characterized as being "short lived" and very little is really known concerning the factors affecting their vitality. Nor do we know the fate of spores on the corn used for ensilage. The present investigation was made in an effort to secure more definite information on these points.

INOCULATION EXPERIMENTS

Methods

Pure cultures of the fungus were obtained by the poured plate method, usually on beerwort agar. The colonies were later transferred to agar in tubes. Sporidia from pure culture were then used in the inoculation experiments. Inoculations were made either by smearing the sporidia directly on to the plant parts or by placing them in water and applying this suspension of sporidia by means of a dropper or hypodermic syringe. The hypodermic syringe was used when it was desired to inoculate the very young parts which had not yet been unfolded.

Spores were also used in inoculating the plants—usually they were dusted directly into the tops of the plants or mixed with moist soil and then applied. In a few cases the spores were applied in suspension in water.

Factors affecting infection

During the summers of 1913 and 1914 a total of 2064 plants of Minnesota no. 13 corn were inoculated. In all eighty different series of plants were used in the tests. The highest percentage of infection in any series in the summer of 1913 was 70.8 and in the summer of 1914, 84.2. These results were obtained by injecting suspensions of sporidia in water into the growing point or as near it as possible.

Experiments were made to determine the effect of the following factors on the success of infection: age of the plants; injury to plants; age of the spores and sporidia. Observations were also made on the relation of early planting close planting and soil conditions to the amount of smut present in the fields.

Age of the plants. It was found that successful infection depended very largely on the age of the plants or plant parts inoculated. Healthy, vigorous plants about 2 to 3 feet high are most susceptible. It was very difficult to infect very young or very old plants. These results confirm those obtained by Brefeld (3) and Hitchcock and Norton (6).

Injury of the plants. Experiments performed by Clinton (4) showed that mutilation of the corn plants when about ready to tassel tends to increase their susceptibility. The experiments of the writer likewise showed that injury tends to increase the chances for infection. When young leaves were injured and then inoculated, the resulting infection usually spread from the point of injury as a center. Many of the infected leaf areas, however, never developed sufficiently to produce mature spores. This was probably due for the most part to the rapid maturing of the leaf tissues and the consequent inability of the fungus to spread through these older tissues. Injury is not necessary, however, for successful infection.

Age of the spores and sporidia. The length of time that the spores can withstand unfavorable conditions is important from the standpoint of the propagation of any fungus. Brefeld (2) demonstrated that corn smut spores, eight years old, germinated, but he did not attempt to secure infection with them. The writer obtained spores, five years old, and made field inoculations with them by dusting into the tops of the plants. Six per cent of the inoculated plants became smutted near the point of inoculation. Two per cent of smut also appeared in the check plot but the infected areas appeared on various parts of the plants.

Many fungi when kept in continuous culture on nutrient media for

some time lose their power to infect. Brefeld (2) found that smut sporidia, when kept for a year in continuous culture in liquid media, lost their viability. The writer kept the sporidia in continuous culture on beer-wort agar for eight months. The culture tubes containing the sporidia were then buried in snow out of doors where they remained for a month. Inoculations were then made in the greenhouse on two corn plants. One, a small plant about 2 feet high, became infected in each of the five leaves inoculated. The other, about 5 feet high, was inoculated on an ear and produced a large smut boil. Thus it will be seen that continuous culture of the sporidia on a solid nutrient medium for a period of eight months, followed by freezing for a month, did not destroy their ability to infect. Recently, portions of a pure culture, that had been maintained on beer-wort agar since June, 1913, a period of three and one-half years, were placed in hanging drops of distilled water and also of modified Cohn's solution. In these cultures individual sporidia were probably no longer present but there was a mass of short hyphal threads, apparently resting segments of the germ-tubes of the sporidia. The ends of these hyphal threads were densely protoplasmic and when they were placed in water, or, better still, in a liquid nutrient medium they again produced sporidia by budding. These sporidia continued to bud until the supply of nutrient material was exhausted when they again sent out long thin germ-tubes. The writer inoculated four plants with some of the pure-culture material but has not as yet succeeded in securing successful infection.

Effect of early planting, close planting and soil conditions. Arthur and Stuart (1) report that early planting, close planting and moist rich soil increase the amount of smut. Rainy periods were also closely followed by outbreaks of smut in the corn fields. The following observations made by the writer point to similar conclusions.

The corn used for the inoculation experiments was planted at successive intervals throughout the season so as to have at all times an abundance of plants in the susceptible stage. The plots were carefully examined for smut and it was found that a greater amount of smut was present in the plots planted earlier in the season. The plants in such fields are in the most susceptible condition at a time when the weather is still fairly cool and moist and the conditions for infection very favorable. The later summer weather is likely to be too hot and too dry for successful infection. Late summer with its more frequent rains and cooler weather again brings on a fresh outbreak of smut, the parts most affected being the rudimentary ears, since the rest of the plant is already too mature.

When corn is grown in closely planted rows for green fodder or silage purposes the smut is also more prevalent, probably because the corn in such fields remains succulent for a longer period of time. In one such

field which had been continuously cropped to corn a number of years, there was 25 per cent of smut as compared with 7 per cent in neighboring fields where the corn was 3 to 4 feet distant in the rows and on which rotation had been practiced. Corn on poorly drained soils or on those which are too dry has less smut than that on a rich, moist soil. The former types of soils produce weak plants that mature rather rapidly, while a moist, rich soil produces a very vigorous succulent plant which apparently is also more susceptible to smut. These observations further confirm the conclusions of other writers.

Character of infection

Smut boils are often so generally distributed on a plant as to lead one to suppose that the infection may have occurred on young plants and that the fungus then spread throughout the growing tissues. It is not uncommon to find a single plant on which the tassel, leaves and the primary and rudimentary ears are smutted while adjacent plants are entirely free of smut. It is therefore difficult to explain why the successive infections should all have occurred on one plant. If the smut were systemic such a phenomenon would be easily explained. Brefeld (3), however, concluded from his inoculations that the infection is local.

Field observations. In order to obtain more complete information on this point, the writer made careful observations on plants artificially inoculated in the field. The results showed that, when successful, signs of infection always appear in from ten to fourteen days after inoculation and that the fungus spreads but little in the tissues. On several plants where the inoculum trickled down the sides of the culm, smut boils were produced at a number of points along the line, involving the leaves, the primary ears and rudimentary ears. In nature, similar conditions might easily arise when a spore falls into the water contained in the funnel formed by the unfolding leaves and produces sporidia. The sporidia could then be easily washed out to the various parts of the plant by rain. This is especially probable since the spores of the smut can germinate as soon as mature and parts beneath the point of primary infection are particularly liable to attack.

Brefeld (3) and Kühn (7) both obtained successful infection on a few seedling plants but in all cases such plants were destroyed by the smut. In order to further ascertain the results of early infection, ten very young smutted plants in a fodder-corn field were selected and marked. All of the plants were about a foot high and showed varying degrees of infection. Of the ten plants under observation eight were killed by the smut in less than a month and the other two were greatly stunted. These two plants,

however, produced healthy ears. Furthermore, many plants that were found smutted when quite young matured healthy ears.

Greenhouse inoculations. It has already been stated that in the field the infection of very young corn plants was very difficult. Further inoculations were made in the greenhouse upon germinating seeds. Forty-three seedlings were dipped into a water suspension of sporidia and then planted in pots. None of the plants developed smut although they were allowed to grow for one and one-half months. These results indicate strongly that infection is purely local and not systemic.

THE VITALITY OF SPORES AND SPORIDIA

It is a well-known fact that corn smut spores retain their viability for a number of years, being extremely resistant to unfavorable conditions. Very little, however, is known concerning the vitality of the sporidia and their resistance to unfavorable conditions has hitherto been supposed to be very slight. The writer also investigated the vitality of the spores and sporidia. The effects of the following factors on the vitality of spores were considered: (1) the silo, (2) temperature, (3) carbon dioxide, (4) acids, and (5) seasonal factors. The influence of the following factors on the vitality of sporidia was considered: (1) temperature, (2) desiccation, (3) acids.

Vitality of spores

Since a considerable amount of corn smut must be carried over into silos with the corn in the process of silo filling, it is important to know the fate of such spores. To determine this, spores were collected in quantity and placed in several silos for various lengths of time. A brief summary of the results is given below.

Samples I. These spores, which had just matured, were collected September 11, 1914, enclosed in a cheesecloth bag and placed about one-third of the way down in a wooden silo. A sample of the same lot was kept as a check in the laboratory at room temperature. The spores were recovered from the silo on November 2, 1914, after having been there about seven weeks. The spores were frozen and were, therefore, thawed out gradually in the laboratory.

Sample II. These spores were collected and placed in a brick silo September 2, 1914. The spores were enclosed in a bag and placed about 15 feet from the bottom of the silo and about 2 feet from the side. They were recovered March 4, 1915, after having been in the silo for over twenty-six weeks. The spores when recovered were frozen and therefore the sample was divided into three portions as follows: (1) A portion

was kept frozen; (2) a portion was kept moist at laboratory temperature; (3) a portion was air dried and kept at laboratory temperature. The object was to eliminate, if possible, the method of handling the spores as a factor which might influence their germination.

Samples III and IV. The spores were collected, placed in bags and buried in a silo October 6, 1915. Sample IV was placed about 2 feet from the side and about 8 feet from the bottom of the silo. It was recovered January 20, 1916, and was kept frozen until February 8, 1916, and then kept dry at room temperature. Sample III, which had been placed in the center of the silo, about two-thirds of the way up, was recovered January 15, 1916. It was also kept frozen until February 8, 1916, when the spores were gradually thawed out and then tested for germination.

TABLE 1

Results of germination tests of spores kept in silo

NUMBER OF TRIALS	SPORE LOT TESTED	MEDIUM IN WHICH TESTS WERE MADE	PERCENTAGE OF GERMINATION
		Sample I	
11	Silo	Distilled water	0
10	Check	Distilled water	25
5	Silo	Modified Cohn's solution	0
5	Check	Modified Cohn's solution	90
6	Silo	Tap water	0
5	Check	Tap water	12.5
		Sample II	
42	Silo	Modified Cohn's solution	0
14	Check	Modified Cohn's solution	90 to 100
10	Silo	Distilled water	0
8	Check	Distilled water	5 to 15
5	Silo	Tap water	0
5	Check	Tap water	15
		Samples III and IV	
18	Silo	Modified Cohn's solution	0
7	Check	Modified Cohn's solution	95 to 100
10	Silo	Sterilized distilled water	0
5	Check	Sterilized distilled water	75 to 95
7	Silo	Tap water	0
3	Check	Tap water	50 to 75
		Sample V	
50	Silo	Modified Cohn's solution	0*
16	Check	Modified Cohn's solution	75 to 95

* One spore germinated.

Sample V. This sample was collected and buried in a silo about 12 feet from the top and 2 feet in from the side on October 4,1915. It was recovered March 16, 1916, thawed out in the laboratory and germination tests were made immediately. The results of the germination tests are summarized in table 1.

The table shows that with but one exception the smut spores did not germinate after having been in the silo. Samples I and II were both tested at the time they were collected, when 68 per cent and 28 per cent, respectively, germinated in water. The single spore which germinated shows a spore may occasionally retain its viability after having been in silage.

The death of the spores may be attributed to a number of possible causes—(1) unfavorable temperatures, (2) gases produced during the changes in the silo, (3) the acids and other chemical substances produced by fermentation, (4) pressure. The effect of each of these factors except the last was tried.

The effect of temperature. The exact changes which accompany the formation of silage from green corn are but imperfectly known. Esten and Mason (5), however, have shown that silage ferments best between 75°F. and 85°F., and that the temperature never rises above 86°F. in properly prepared silage, except in the topmost layers where destructive fermentation occurs. Neidig (8) reports a maximum temperature of 91°F. Such temperatures are not in themselves sufficient to kill corn smut spores, since Stewart (9) found that a temperature of 52°C., for fifteen minutes was necessary to kill the spores when immersed in water. He also found that exposure to dry heat between 105.5°C. and 106°C., for fifteen minutes killed the spores.

In a few tests made by the writer some spores withstood a dry heat of 103°C. for five minutes, but as a rule a temperature of 100°C. for five minutes is sufficient to destroy the germinating powers of the spores.

Freezing temperatures do not seem to injure the spores as they survive our severest winters. Spores, stored in a shed where they were merely sheltered from snow and rain but were exposed to all the rigors of winter, germinated well in spring. Spores that were frozen for short periods of time and then tested for germination appeared to have been stimulated by the cold. The extremes in temperature which the spores encounter in the silo, therefore, can have no influence on their vitality.

The effect of gases. Little is known of the gases produced in silage formation although it is not probable that they would take an active part in the destruction of the smut spores. Carbon dioxide is probably produced in greatest quantity as a result of the fermentative action. A single experiment was made to determine the effects of the gas on the spores. Dry spores were placed in a bottle and carbon dioxide was passed in. The

spores were thus exposed to the gas for ten days, when germination tests were made. The spores were not only unharmed but germinated much more quickly than those of the same lot which were used as checks. At the end of two days no difference was apparent in the germination of the two lots of spores.

The effects of acids. It seems probable that the loss of viability of spores may be due to the chemical substances produced in silage. Considerable quantities of acids are produced rapidly, the maximum amount of each usually being produced within two weeks after the silo is filled. The total acidity of silage, according to Esten and Mason (5), is about 1.0 per cent to 1.5 per cent, the principal acids in order of their importance being lactic, acetic and propionic. In experiments to ascertain the effect of certain of these acids on the germination of smut spores it was found that a concentration of 1 per cent of either acetic or lactic acids or a combination of the two was sufficient to inhibit smut spore germination. However, spores germinated in a diluted sample of normal silage juice.

The above results are significant in that they indicate to some extent what happens to the spores in the silo. The optimum for the germination of spores is reached at some point in the rise of temperature which accompanies the formation of silage. The germination of the spores is, however, inhibited by the presence of the acids. Acetic acid penetrates rapidly and kills plant tissues. Hence, it is not unlikely that the spores, which are exposed to its action for a long time, are killed by it.

Seasonal factors. Fresh spores were collected from time to time and observations made on their germination. The first tests were made in the summer of 1913 and fresh spores germinated very readily in water at room temperature. The tests made in the summer of 1914 were more complete, beginning with the very first smut spores produced in the field. The first germination test of fresh smut spores was made June 24, the last test on October 10. Fifteen distinct tests were made, the results showing conclusively that fresh spores germinate readily in water. In fact, fresh spores germinated much better than did spores from the same lot kept until winter. The average percentage of germination for the entire series was 42.8, the percentages in different hanging drops varying from 0 to 85, a result often obtained when water is used as the medium for germination. This shows that spore germination in water is somewhat capricious, thus probably explaining the conflicting results obtained by various investigators. The germination in sugar solutions and liquid nutrient media, especially modified Cohn's solution is more uniform, 100 per cent of the spores almost always germinating. No difference could be found in the germination in sterilized distilled water, distilled water, tap water or rain water. Incubating the cells at 24°C. to 38°C. did not seem to influence the rate or amount of germination of the smut spores.

Vitality of sporidia

Methods. In the studies of sporidia, pure cultures of the smut were used throughout. Spores were sown in poured plates of beerwort agar. In about two days the spores germinated and the position of the colonies was marked. At the end of four or five days when the colonies were about the size of a pinhead, they were transferred to beerwort agar slants. A number of other nutrient media were also tried, viz.: carrot agar, nitrogen free agar, beef agar, oat agar and a synthetic agar. Of these, beerwort agar was found to be the best, although a good growth was also obtained on carrot agar.

Cultural characters and morphology. When the colonies first appear they are round, raised, convex, opaque, slightly shiny to dull, light cream in color. As the colonies grow older, the edge becomes somewhat lobed and irregular, and the surface becomes convoluted, ridged, or sharply papillate. The color deepens with age, becoming light lavender in old cultures. The consistency is first soft and ropy, then becomes mucilaginous or rubbery; or, when kept moist, butyrous.

The colonies consist of sporidia which are abjointed from the sides and occasionally from the end of the promycelium. These sporidia are of the same nature as those produced beneath the surface in liquid media. The sporidia produced in the air from a liquid culture are small, sharply fusoid and fairly thick walled. They are produced in long chains. Those produced within the liquid or on solid nutrient media are larger than the air conidia, not as thick walled and are somewhat rounded at the ends. They are plumper, contain more oil globules and are not produced in such long chains. The walls are apparently somewhat mucilaginous. In continuous culture the sporidia produce long germ-tubes, the ends of which are densely protoplasmic and which, therefore, can become resting segments possessing all the properties of sporidia. The germ-tubes become much entangled and give the culture its rubbery consistency, while the disintegration of the empty portions of the hyphae give it its mucilaginous character. A smear from such a culture dries almost instantly and becomes brittle. While sporidia produced in culture may not be exactly like those produced in nature, still they must be very similar to those which we imagine are produced in such great abundance in manure.

Thermal relations. A complete understanding of the thermal relations of corn smut sporidia would not only be of value in throwing additional light upon the phenomena of infection, but it would also show more clearly the optimum conditions for the propagation of the fungus. An attempt was therefore made to ascertain the minimum, maximum and optimum temperatures. Both dried and actively vegetating sporidia were tested.

At temperatures ranging from 20°C. to 26°C. the small sporidia bud

profusely in nutrient solutions. As the temperature is increased to above 26°C. the sporidia show a greater tendency to produce long, slender germ-tubes. At 35°C. growth is somewhat inhibited and the cells begin to show an increase in the number of vacuoles. Increasing vacuolation continues with a rise in temperature to 40°C. where growth practically ceases, while at 46°C. the cells are no longer alive.

Attempts to determine the lowest temperature which the sporidia in liquids or on solid nutrient media can endure gave negative results because they withstood the severest cold of the winter (about −28°C.). Alternate freezing and thawing, however, kills moist sporidia. Desiccated sporidia, on the other hand, were not only able to withstand severe freezing but in some cases were not severely injured by alternate freezing and thawing.

When exposed to alternate freezing and thawing, however, there seems to be some injury, as no subsequent growth occurred in two out of three tests. Smears direct from pure cultures were not killed by drying for one day at 21°C. or for fourteen days at a temperature of from 7° to 9°C.

TABLE 2

The effect of temperature on desiccated sporidia of Ustilago Zeæ. All sporidia dried at 21°C.

TEST NUMBER	NUMBER OF TRIALS	NUMBER OF DAYS DRIED	TEMPERATURE TO WHICH EXPOSED	TIME EXPOSED	MEDIUM FOR GERMINATION	RESULTS*
1	1	1	−10°C. to −7°	14 days	Sterilized distilled water	+
2	1	5	43°	15 min.	Sterilized distilled water	+
3	1	6	Alternate freezing and thawing	31 days	2 per cent sugar solution	−
4	1	7	−2°C. to 3°	12 hrs.	Sterilized distilled water	+
5	1	12	45°	15 min.	Sterilized distilled water	+
6	1	16	28.5°C. to 31°	24 hrs.	Sterilized distilled water	+
7	2	16	−7	12 hrs.	Sterilized distilled water	+
8	1	18	54°C. to 55°	15 min.	Sterilized distilled water	−
9	1	19	40°C. to 50°	16 hrs.	Sterilized distilled water	±
10	1	20	−5°C. to 1° alternate freezing and thawing	12 days	Sterilized distilled water	+
11	1	20	−5C°. to 1° alternate freezing and thawing	12 days	Sterilized distilled water	−
12	1	20	28.5°C. to 31°	24 hrs.	Sterilized distilled water	±

* No growth −; grew weakly ±; grew +.

But sporidia dried six days and then exposed to alternate freezing and thawing for thirty-one days were killed.

Sporidial smears direct on glass cover-slips from the pure culture were unaffected by drying for sixteen days at 21°C. and then at 28.5° to 31°C. for one day. Sporidia first placed in water, then dried for twenty days, also withstood the same temperature. Sporidia in smears were not killed by exposure to from 40° to 50°C. for sixteen hours, after drying for nineteen days, but appeared to be killed at 54° to 55°C. for fifteen minutes,

TABLE 3

The effect of desiccation on the vitality of sporidia of Ustilago Zeæ. All tests for germination made at 21°C.

TEST NUMBER	NUMBER OF TRIALS	METHOD OF PREPARATION	NUMBER OF DAYS DRIED	MEDIUM USED FOR GERMINATION	RESULTS*
1	2	Grown in cells in H_2O 2 days. Slip dried	3	Sterilized distilled water	++
2	3	Spores germinating in H_2O in cell. Slip dried	3	Sterilized distilled water	+
3	5	In H_2O in cells. Slip dried	1	Sterilized distilled water	+
4	1	Smears made on slips	12	2 per cent sugar solution	+
4	1	Smears made on slips	56	Modified Cohn's solution	+
4	1	Smears made on slips	6 at 21°C. 31 at alternate freezing and thawing	2 per cent sugar solution	−
5	1	In water on slips	12	2 per cent sugar solution	+
5	2	In water on slips	48	Modified Cohn's solution	−
5	1	In water on slips	20 at 21°C. 24 hrs. at 28.5°–31°C.	Sterilized distilled water	±
6	1	Smears on slips	7	2 per cent sugar solution	+
6	1	Smears on slips	51	Modified Cohn's solution	+
7	1	Smears on slips	16 at 21°C. 24 hrs. at 28.5°–31°C.	Sterilized distilled water	+
7	1	Smears on slips	1 day at 21°C. 14 days at 15°–20°C.	Sterilized distilled water	+
8	3	In water on slips	124	Modified Cohn's solution	−

* ++ denotes very abundant germination; + abundant; ± sparse; − no germination.

TABLE 4

Viability of sporidia of Ustilago Zeæ when smeared on a cover slip and desiccated in light and darkness. All germination tests made in sterilized distilled water at a temperature of 21°C.

NUMBER OF TRIALS	CONDITION UNDER WHICH DRIED	DAYS DRIED	RESULTS
2	Light	1	+
2	Dark	1	+
1	Light	1½	+
1	Dark	1½	+
1	Light	6	+
1	Dark	6	+
1	Light	8	+
1	Dark	8	+
1	Light	11	+
1	Dark	86	+
1	Light	87	+
1	Dark	149	+

after drying for eighteen days. The thermal death point of dried sporidia is, therefore, probably about 54° or 55°C.

Moisture relations. In most cases the sporidia were taken directly from the pure cultures on beerwort agar and smeared on sterilized coverslips. The slips were then placed in sterilized petri dishes and were allowed to dry at room temperature for various lengths of time. In other cases a water suspension was first made of the sporidia and drops of this were then transferred to the slips and allowed to dry as above. When the germination test was made a drop of the desired medium was added and the slips were then mounted on Ward or Van Tieghem cells.

From the results shown in tables 3 and 4 it is apparent that sporidia can withstand long periods of drying without serious injury. Sporidia when taken directly from pure cultures withstood drying for 149 days at room temperature. Not all of these sporidia, however, remained viable. Sporidia first placed in water and then dried seemed to be less resistant to desiccation. Sporidia thus treated grew after drying for twenty days, but not when dried for forty-eight days. The latter result may be somewhat misleading as the number of sporidia in a water drop is much smaller than the number in a smear from pure culture.

There was no noticeable difference between sporidial smears dried in the dark and in the light. Light, therefore, is probably not very injurious to sporidia.

The above results are not in accord with those of Brefeld (3) who found that sporidia were killed when dried five weeks. Nor are they in accord with the statements of Arthur and Stuart (1) who characterize the sporidia

as "short lived" and further add, "These are borne through the air which must be rather moist or the sporidia will be killed by drying."

Chemical relations. Sporidia were placed in various solutions of acetic and lactic acid, in corn silage juice, and in a mixture of various acids in such proportion as to approximate the composition of silage juice. If corn smut spores germinate when placed in a silo, it was thought that by means of tests with various acids commonly produced in silage the fate of the sporidia might be determined. The sporidia in each case were obtained directly from pure cultures on beerwort agar and were transferred to the solution from which hanging drops were then made.

Sporidia apparently can grow in 1 per cent acetic or lactic acid solutions or in a mixture of the two. In the lactic acid the sporidia tend to produce a greater number of germ-tubes which probably indicates that the medium is slightly unfavorable for growth. The sporidia also grow well in expressed silage juice. It seems probable, therefore, that if spores do germinate in the silo, the sporidia may continue to live in the silo for some time. Whether long exposure to the action of the acids would be detrimental or not was not determined.

A more concentrated mixture of acids such as was used in the silage acid test proved to be deleterious to the growth of the sporidia. The sporidia appeared starved and became greatly vacuolated, a condition which probably precedes their death. The results here obtained are not in accord with those obtained when silage juice itself was used, possibly owing to the lack of sugars or other nutrients in the silage acid mixture. The effect of the traces of butyric and propionic acids alone upon the sporidia has not yet been determined.

SUMMARY

1. The infection of corn by *Ustilago Zeæ* (Beckm.) Unger is purely local; no evidence of systemic infection was obtained.

2. When very young plants become infected they are often killed.

3. Injury to the host plant, close planting, very early or very late planting, and growth on rich soil are conducive to heavy smut attacks.

4. Vigorously growing plants, between two and three feet high, are most susceptible to smut attack.

5. The spores of *U. Zeæ* can cause infection either when young or old. Spores germinate readily as soon as mature and retain their viability for several years; infection was obtained by inoculating corn plants with spores five years old.

6. The corn-smut fungus does not lose its virulence quickly when grown on artificial media.

7. The spores of *U. Zeæ*, almost without exception, lost their viability after having been kept in a silo for a few weeks.

8. The factors causing spores to lose their viability in the silo have not been determined definitely; it seems probable that the silage acids, especially acetic, may be the destructive agents.

9. Sporidia were kept in pure culture continuously for three and a half years, at the end of which they remained viable. Inoculation experiments with the same material gave inconclusive results.

10. Sporidia were desiccated for about five months without seriously impairing their vitality.

11. Freezing injures sporidia but little; alternate freezing and thawing, however, is injurious to moist sporidia, less so to desiccated sporidia.

12. The optimum temperature for the budding of sporidia is between 20° and 26°C., the maximum at about 40°C. and the thermal death point near 46°C.

13. Sporidia can germinate and bud in silage juice, but are injured in a solution containing acids in the proportionate concentration in which they occur in silage.

14. The ability of sporidia, as well as spores, to withstand unfavorable conditions is very significant in explaining some of the facts in the parasitism of *U. Zeæ*.

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SCLEROTIUM BATATICOLA

The Cause of a Fruit-Rot of Peppers

William H. Martin

During the past few years the writer's attention has been called a number of times to a rot of peppers. Specimens were sent in from various sections of New Jersey with inquiries as to the nature of the trouble. Except for a drying and shrivelling of the epidermis in the more advanced stages, there was little external evidence of the presence of the disease. On breaking the fruit open, however, the interior was found to contain numerous, small, black sclerotia which were also present on the seed. Isolations were made, and in all cases pure cultures of an organism resembling *Sclerotium bataticola* Taub. was secured. The marked similarity of the two organisms led to experiments to establish their pathogenicity as well as to determine if they were identical.

The fungus was isolated by aseptically breaking the diseased fruit; bits of tissue were then picked out with a sterilized needle and transferred to a poured plate of nutrient agar. In most cases pure cultures were obtained at the first planting. Inoculations were made as follows: Healthy pepper fruits were immersed for ten minutes in a 1 to 1000 solution of mercuric chlorid, washed in sterilized, distilled water and placed in a sterilized moist chamber. Attempts to produce the disease by placing bits of the culture media together with sclerotia on the unbroken surface failed. White tufts of mycelium were formed but the fungus seemed unable to penetrate the epidermis. However, inoculations on a cut surface made with a flamed scalpel were uniformly successful. A large number of inoculations were made in this manner and 100 per cent infection resulted. Infection was evident in from four to seven days. The epidermis at the point of infection became blackened and the rot spread throughout the interior. After the fungus became established, its progress was rather rapid; in two weeks the entire interior of the fruits were invaded and numerous sclerotia developed in the tissue and on the seed. Except for a blackening of the epidermis there were no external signs to indicate the presence of the disease. Inoculations were also made on fruit still attached to the plant and in every case the results were positive.

Attempts were made to inoculate the roots and stems of growing plants. The soil was carefully removed from around the roots and an incision was

made with a sterilized scalpel and sclerotia from young cultures were inserted. The soil was then replaced. Roots similarly treated, but not inoculated, were used as checks. Stems were likewise inoculated and the cut surfaces were wrapped with cotton. On the stems and roots the inoculations were not as successful as on the fruits. Where infection did occur the fungus was found just beneath the epidermis or in the pith. Death of the plant or plant part resulted before the mycelium had advanced far from the point of inoculation.

Reisolations were made from both the fruit and stem and the fungus was again grown in pure culture. Inoculations with these reisolated cultures were, in every case, successful.

During the process of the above inoculations, similar inoculations were made on peppers with cultures of *S. bataticola* Taub. isolated from the sweet potato. In every instance they were successful. Not only was this true but the characteristics and growth of the organism were, in all respects, like the pepper Sclerotium.

In order to more fully prove the identity of these two pathogenes, the following comparisons were made: (1) Growth on culture media, (2) cross-inoculations on sweet potato and pepper as well as other hosts, (3) development, measurements and the external and internal appearance of sclerotia.

Growth comparisons were made on corn meal agar, potato agar, bean plugs and nutrient agar. Abundant growth was made and no differences were observed.

For the cross-inoculations three strains of the organism were used. One was isolated from the sweet potato, another, secured from Dr. J. J. Taubenhaus, was likewise isolated from the sweet potato. The third was isolated from peppers. These cultures may be designated 1, 2, 3, respectively. The methods employed in the inoculations were the same as have been previously described. The following inoculations were made:

TABLE 1

Results of inoculations on various hosts with three strains of Sclerotium bataticola

HOST	NUMBER INOCULATED WITH EACH CULTURE	NUMBER OF CHECKS	PER CENT INFECTION
Sweet potato (Ipomœa batatas)	25	10	100
Pepper (Capsicum annuum)	25	10	100
Tomato (Lycopersicum esculentum)	3	2	100
Cucumber (Cucumis sativus)	3	2	100
Apple (Pyrus malus)	3	2	100
Eggplant (Solanum melongena)	1	1	100
Turnip (Brassica campestris)	3	2	100
Red beet (Beta vulgaris)	3	2	0
Parsnip (Pastinaca sativa)	3	2	0
Carrot (Daucus carota)	3	2	0

In those cases where the inoculations were successful, no differences in growth of the organism were evident. The time before infection is evident, varies, however, with different host plants. On pepper, cucumber and tomato from four to seven days is required, while on sweet potato, apple, turnip and eggplant, from three to seven weeks elapses.

The development of sclerotia was followed in Van Tieghem cells. The method employed was to pour enough of a melted nutrient medium on a cover glass to form a thin film. After this had cooled, it was inoculated and inverted over the glass ring. If care is taken in this procedure no contamination results. By this method it is easily possible to trace the development of a single sclerotium under high magnification of the microscope.

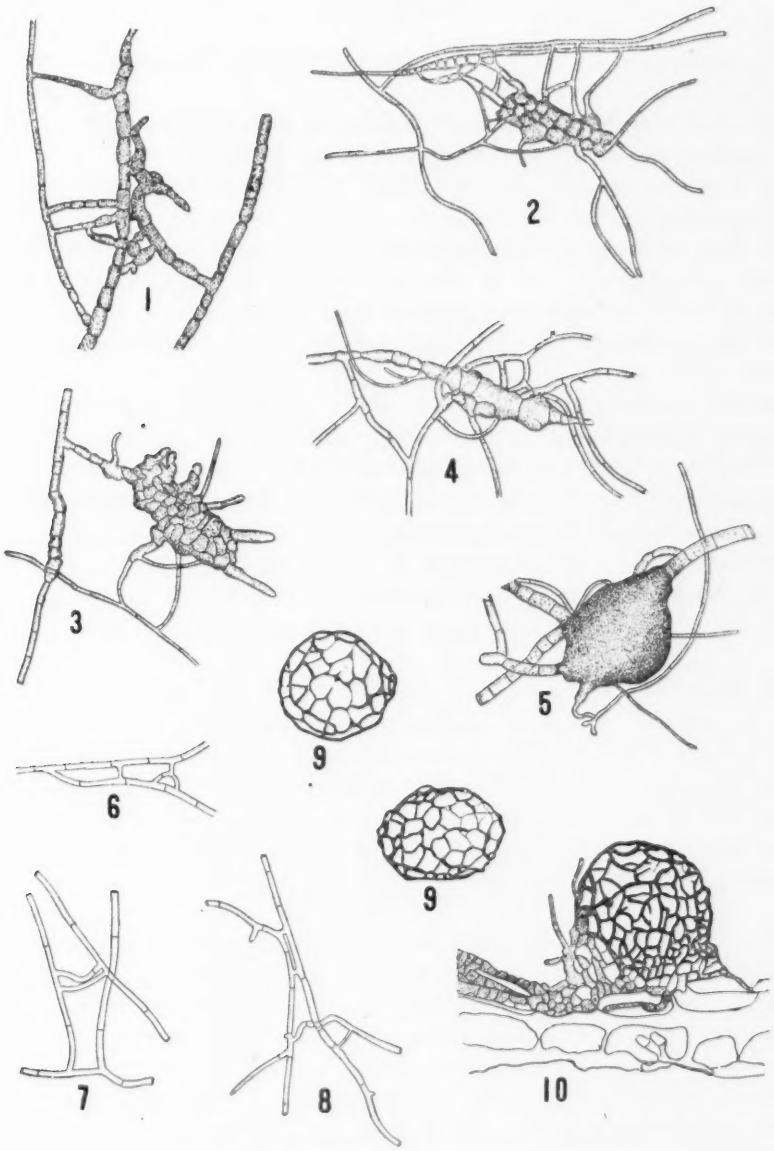
Some cases were noted where sclerotia appeared to have developed from a single mycelial strand; in the majority of cases observed, however, several or a number of strands were involved. Sclerotial development is preceded by the formation of many septa. Short mycelial tubes are formed which connect the strands. These in turn become very much septate, as do the radial hyphae which are formed later (fig. 1). As the sclerotium enlarges the central cells become contorted and form a compact mass, due perhaps to the pressure or resistance of the outer cells (figs. 2, 3, 4). The mature sclerotia are coal-black in color and in most cases are free from any surface irregularities (fig. 5). No differences were observed in the formation or color of sclerotia of the different strains.

In the progress of these studies numerous instances were observed where mycelial branches united; this took place between branches of a single strand as well as between two separate strands (figs. 6, 7, 8). In some few cases, this was the first step in sclerotial development, but usually the mycelium becomes septate and irregular.

Sections of the sclerotia of the different strains showed them to be alike with regard to their internal structure (figs. 9 and 10). In this connection seeds were sectioned to determine if the mycelium penetrated the seed coat, but in none of the cases examined was this observed to be true. The appearance of sclerotia on the seed did not appear to affect germination. Healthy plants were grown from diseased seed. Measurements of the sclerotia gave results identical with those of Taubenhaus.[1]

Attempts to produce a perfect stage failed. In every case, on the germination of the sclerotia new sclerotia were formed.

[1] J. J. Taubenhaus. The black rots of the sweet potato. Phytopath. **3**: 159–163. 1913.



SCLEROTIAL DEVELOPMENT OF SCLEROTIUM BATATICOLA

FIGS. 1, 2, 3, 4 and 5. Stages in sclerotial development.
FIGS. 6, 7 and 8. Anastomising of hyphae.
FIG. 9. Sections through mature sclerotia.
FIG. 10. Section of sclerotium on epidermis of pepper fruit.

CONCLUSIONS

The pathogenicity of *Sclerotium* sp. causing a rot of peppers (*Capsicum annum* L.) has been established.

The following facts warrant the conclusion that the sclerotium occurring on peppers is identical with *Sclerotium bataticola* Taub.

a. Cross-inoculations on sweet potato and pepper as well as other hosts gave positive results.

b. With both strains the growth was identical both in culture and on the host.

c. Measurements of the sclerotia are identical.

d. Sclerotial development, color and structure is the same with both strains.

It appears from these studies that the charcoal rot of sweet potatoes (*Ipomœa batata*) is common and widely distributed throughout sections of New Jersey, that *S. bataticola* Taub. can probably persist on several other hosts and that it is the cause of a disease of minor importance of peppers.

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A NECTRIA PARASITIC ON NORWAY MAPLE

MEL. T. COOK

During the summer of 1913 the writer's attention was called to an interesting disease on a group of Norway maples growing on the private grounds of one of the residents of Princeton, N. J. The first symptoms of the disease were the wilting of the leaves and dying of branches very similar to the well known symptoms of the chestnut bark blight disease, caused by *Endothia parasitica*. In fact, it was the very striking resemblance to the chestnut blight that attracted the attention of the superintendent of the grounds and led to the writer's being called to make an examination. A further examination of the dead branches showed a still more marked resemblance to the chestnut bark blight; the dead branches had been completely girdled by a canker which showed a blackening and slight sinking of the diseased bark. The older cankers were covered with the orange-colored pustules of the Tubercularia stage of a Nectria and there was abundant evidence that this organism was the cause of the trouble.

The superintendent had been watching the disease for some time and had removed many dead branches and cankers. Fresh wounds were very quickly attacked by the fungus, which made its first growth in the oozing sap, but the careful treatment of these wounds with antiseptics, followed by an application of paint, greatly reduced the number of infections. The breaking of the small lateral twigs from the trunk and larger branches was the most common source of natural infections and most of the cankers had started from wounds of this kind.

An inspection of the trees on the estate and in the immediate vicinity showed two others badly affected. These were destroyed, but the clump previously referred to was left standing for observation and study. During the remainder of 1913 the infected parts were removed as soon as detected and the wounds treated. In the fall of 1913 and the spring of 1914 heavy applications of fertilizers were applied to all the trees and observations continued during the summer of 1914. The disease reappeared and the fungus was always present on the cankers, but was much less severe than in 1913. The fungus was frequently found in the dead bark around old wounds, but in many cases did not appear to be parasitic. About one-third of the trees in this clump showed the fungus in 1914. Another clump of trees on the opposite side of the driveway showed but

very little of the fungus. The clump of trees has been kept under observation during 1915 and 1916, and the disease, although present is much less severe than in 1913. Wounds are frequently infected but the increased vigor of the trees, and the careful removal of diseased branches as soon as detected, has apparently resulted in a great reduction of the disease.

Since 1913 the writer has frequently found the fungus on Norway maples and also on the mulberry, working saprophytically and also apparently as a weak parasite.

The idea that Nectria is parasitic is not new in either Europe or America. Wehmer[1] reports having found it on the healthy stumps of a cut-over thicket of trees and shrubs, especially on *Carpinus* sp. He also found a Diplodia and a Tubercularia on the young twigs of walnut, sometimes associated and sometimes the Tubercularia growing alone. He states that the twigs were winter-killed, and that the fungus gained access to the host through the dead parts from which it worked its way into the living tissues. The injured twigs were defoliated and the tree bore very little fruit.

It was also reported by Behrens[2] as attacking *Abies balsamea*. The terminal buds were frequently swollen. This was due to the formation of a layer of cork between the healthy and necrotic tissues. The swellings of the previous year were mostly dead. Some of the green twigs did not develop their buds and some died later. The mycelium hibernated in the dead wood and penetrated the living wood the following season.

The most important American report is by Pollock[3] who found *Nectria coccinea* (Pers.) Fr. causing cankers on yellow birch. These cankers frequently girdle the infected parts. In case of infected twigs hypertrophies were frequently formed. Several American students have reported similar observations to the author.

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PHYTOPATHOLOGICAL NOTES

Notes on Razoumofskya campylopoda. Berries of the false mistletoe, *Razoumofskya campylopoda* (Engelm.) Piper, growing on *Pinus sabiniana*, were collected by Dr. E. P. Meinecke in the San Rafael Mountains, Santa Barbara County, California (Forest Pathology No. 17026), November 12, 1914, and sent to the writers. Seeds from these berries were used November 19, 1914, for inoculating young pine trees in pots as follows: one *Pinus banksiana* Lamb., one *P. bungeana* L., four *P. caribaea* Morelet, four *P. contorta* Loud., two *P. coulteri* Lamb., one *P. densiflora* Lieb. & Zucc., two *P. halapensis* Mill., six *P. mayriana* Sudw., two *P. monophylla* Torr. & Frem., one *P. nigra* Arnold, two *P. parviflora* Lieb. & Zucc., six *P. pinaster* Ait., one *P. pinea* L., one *P. resinosa* Ait., two *P. rigida* Mill., four *P. sabiniana* Dougl., one *P. strobus* L., and two *P. virginiana* Mill. Two trees of *Larix occidentalis* Nutt. and two of *Pseudotsuga taxifolia* (Lam.) Britton were also inoculated. The trees used were from three to six years old. The seeds, enclosed in pulp, were placed chiefly in the axils of the leaves on the younger portions of the shoots, adhering firmly as soon as the pulp dried. Many germinated, but the radicles of only a few succeeded in penetrating the bark of the trees on which they were borne. In six months plants became established on the following species of trees: one *Pinus banksiana*, one *P. bungeana*, one *P. caribaea*, one *P. pinea*, two *P. sabiniana*, and two *P. virginiana*. On *Pinus bungeana* and *P. virginiana*, dense witches-brooms formed around the mistletoe infested region. On the other species spindle-shaped swellings without witches-brooms were usually produced at the point of attack. All these trees except *Pinus sabiniana* are new hosts for this species of mistletoe in this country.

All the trees inoculated successfully produced clusters of mistletoe plants in 1916, none of which produced mature fruits, apparently owing to lack of fertilization. In 1917 mistletoe plants are again developing on all trees except those with dense witches-brooms.

The effect of the mistletoe is to stunt appreciably the growth of all the trees inoculated, as compared to other similar trees of the same species not inoculated. Only one of the trees successfully inoculated has died from this effect after two years' growth, one of *Pinus virginiana* with a witches-broom. In case of trees of the same species inoculated under similar conditions with *Peridermium cerebrum* Peck, and *P. harknessii*

Moore, nearly 50 per cent of the infected trees died inside of two years, indicating that the stem rusts are much more destructive to young pines than the false mistletoes.

The mistletoe is a western species which grows vigorously on eastern species of pines. Since the infected areas on young pines may not be conspicuous during the first season's growth and because of the fact that the aerial parts of the mistletoe plants are annual, and are not usually observed on dormant trees, inspection of nursery stock is not sufficient to insure its freedom from this harmful parasite. Shipments from the Rocky Mountain and Pacific regions to those farther east should be discouraged, as they are likely to carry the mistletoe, even though they may appear clean. Our eastern pines are at present free from mistletoes, and should remain so.

GEO. G. HEDGCOCK AND N. REX HUNT

The production of spores by Alternaria Solani in pure culture. This fungus has been the subject of special study by the author at the University of Wisconsin during the past three years. The scarcity of spore production in pure cultures, which has been noted by previous workers, was at the outset a hindrance to inoculation experiments. Trials with twenty different kinds of media including some tests on the effect of variation of acidity and temperature were made, but the result was always the same, few spores being produced. In February, 1915, experiments were begun in which the moisture content of the medium and the humidity of the air above the culture were varied. This likewise was without results except when the culture was shredded and the mycelium severely wounded. Under such treatment enormous sporulation was always secured (fig. 1, *B*). A few spores were formed when the mycelium alone was cut. The most successful method consists in growing the Alternaria in petri dish culture, on hard potato agar, for ten to twelve days, then, first, shredding the agar to bits (fig. 1, *A*) and stirring to separate and evenly distribute the pieces; second, controlling for twenty-four to forty-eight hours thereafter the moisture relation so that partial drying out is effected without allowing the more exposed surfaces to become desiccated and hard. The latter object is accomplished most readily by removing the lid and exposing the dish to sunlight in a sterilized moist chamber or bell jar. If evaporation is too rapid, occasional atomizing with sterilized water is necessary. Spores were obtained by this method in total darkness in the incubator at 26° C. but closer attention was required.

Over the cut and exposed surfaces of the agar there develops from the old mycelium a network of closely septate, thick-walled hyphae, from which

the conidiophores arise. The initial stimulus for sporulation seems to be largely the result of the wounding of the mycelium in connection with changes in the vapor tension of the air in which it is exposed. After the spores have been rinsed off, a second and even a third crop may be ob-

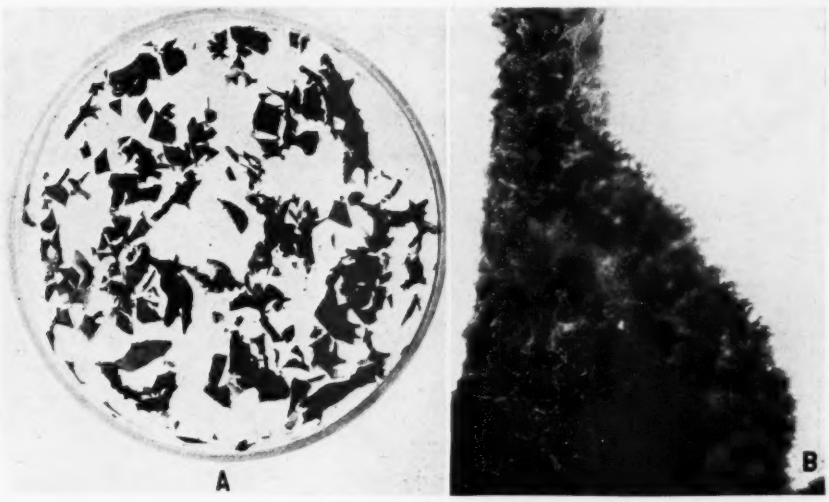

FIG. 1. ALTERNARIA SOLANI

A, Photograph of shredded culture. *B*, Fragment of same magnified × 32 to show abundant sporulation.

tained by moistening the surface and exposing as above. Measurements indicate greater uniformity in size of spores thus produced than on the spots in nature. This method may prove useful with some other fungi which sporulate poorly in culture.

R. D. RANDS

Lightning injury to sugar cane. In a recent note (Phytopath. **7**: 140–142) the lack of reports of lightning injury to any of the Gramineæ was noted. In this connection it may be of interest to record an instance of this kind observed by the writer.

During the past year there was called to our attention a spot in one of the sugar cane fields at the Insular Experiment Station, Rio Piedras, Porto Rico, in which the cane had been completely killed out. The area was nearly circular and approximately a rod in diameter, sharply set off from the surrounding cane, which at the time the first observation was made was at its full height.

In the area itself only a few dead and dry stalks, some broken off 2 or 3 feet above the ground level and some prostrate, remained. There had been no growth of new shoots, and examination showed that the underground portions of the stools were likewise dead. There was a growth of weeds present but entirely of herbaceous annuals, which could have been seeded from the margin of the area. No signs of insects, rodents, or fungi were found either above or below ground, not even of the common saprophytic forms so common on dead and dying canes, of which some eighty are known. The surrounding cane was likewise entirely free of insects or fungous diseases and normal in all respects.

FIG. 1. LIGHTNING INJURY TO SUGAR CANE

Charred bits of cane trash were finally discovered, which fact, combined with the other observations, indicated that lightning was the cause. No other source of fire could have destroyed green cane so completely, especially the underground portions of the stools. The burning over of cane fields is a very common practice in Porto Rico, and fields so treated have been observed in innumerable instances, so that the possibility of an ordinary surface fire through a carelessly dropped match or other source having been the cause need not be considered.

Occurrences of this kind are apparently rare.

JOHN A. STEVENSON

Phytophthora infestans, causing damping-off of tomatoes. *Phytophthora infestans* has been recorded as occurring on tomatoes by both American and European pathologists. So far as literature on the subject is available to the writer, no one appears to have noted this organism as causing damping-off of young tomato plants. The following brief account of a severe outbreak of damping-off of tomatoes caused by *Phytophthora infestans* may be of interest therefore to pathologists.

In June, 1916, a number of young diseased tomato plants were received by the Ontario Agricultural College from J. W. Noble, Essex, Ontario. In the letter accompanying the plants Mr. Noble stated that thousands of late tomato plants in that district had been destroyed. The affected plants were first observed shortly after the tomatoes were set in the field.

A glance at the plants revealed brown lesions and constrictions on the stems near the surface of the ground. Many of the plants showing these symptoms had fallen over, due to the collapse of the stems at this point. On examining the stems with a hand lens a white fungous growth was clearly seen on the lesions. This when examined under the microscope proved to be the conidiophores and conidia of *Phytophthora infestans* (Mont.) deBary. After this all the plants were examined very carefully, and on some of them the same fungus was found, apparently causing a blighting of the leaves; but the chief damage done to all of them was by the destruction of the stem near the surface of the ground. The falling over of the plants by hundreds in the field was what first brought the disease to the attention of the growers.

Some idea of the severity of this outbreak of damping-off of tomato plants may be had by considering the fact that out of 288,175 tomato plants supplied by the Heinz Pickle Company, Leamington, Ontario, to Pelee Island growers, only 45,000 reached maturity. At least 50 per cent of the plants that did not survive succumbed to damping-off due to *Phytophthora infestans.*

It is interesting to note that weather conditions during June were exceptionally favorable to the spread and development of *Phytophthora infestans,* the rainfall being much above the average for June, and the temperature relatively low.

J. E. HOWITT

State and National quarantines against the white pine blister rust. The following table shows the State and National quarantine action taken to date against the white pine blister rust. The action is so varied in character that it seems necessary to present it in this form. Similar action is under consideration in a number of other states.

STATE	WHITE PINES	RIBES, GROSSULARIA	DATE	QUARANTINED AREA
Canada.....	All	None	November 14, 1914	All foreign countries
United States...	P. strobus, monticola, lambertiana, cembra	None	September 16, 1912	Great Britain, France, Belgium, Holland, Denmark, Norway, Sweden, Russia, Germany, Austria, Italy, Switzerland
	All	None	May 21, 1913	Europe and Asia
	All	All	March 16, 1916	Canada and Newfoundland
	All	All	June 1, 1917	All points east of, and including the states of Minnesota, Iowa, Missouri, Arkansas, Louisiana
	All	R. nigrum	June 1, 1917	States of New England and New York
	None	All	June 1, 1917	Europe and Asia
California.....	All	All	February, 1917	East of Mississippi River
Delaware.....	All	All	March 2, 1917	All points outside state
Idaho.....	All	All	March 1, 1916	New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania
Indiana.....	All	All	March 13, 1917	All points outside state
Kansas.....	All	All	March 10, 1917	All points outside state
Massachusetts..	All	None	June 1, 1912	Europe
Michigan.....	All	All	March 19, 1917	All points outside state
Minnesota.....	All	None	April 30, 1917	Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, New York, New Jersey, Pennsylvania, Ohio, Wisconsin
Montana.....	All	All	July 17, 1916	New Hampshire, Vermont, Massachusetts, Connecticut, New York, Pennsylvania
Nevada.....	All pines	All	March 10, 1917	East of Mississippi River and Minnesota; all foreign countries
New Jersey.....	All	None	April 16, 1917	Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, Pennsylvania, New York, Minnesota, Wisconsin

STATE	WHITE PINES	RIBES, GROSSULARIA	DATE	QUARANTINED AREA
New York.....	All	None	March 24, 1917	Ohio, Indiana, Minnesota, Wisconsin, Maine, New Hampshire, Vermont, Massachusetts, Rhode Island, Connecticut, Pennsylvania, Illinois, New Jersey
Ohio.....	P. strobus, monticola, lambertiana, cembra, excelsa, flexilis	None	February 21, 1917	All points outside state
Oregon.....	All	All	July 24, 1916	East of Mississippi River; all foreign countries
Pennsylvania...	All	None	March 12, 1917	All points outside state
South Dakota...	All	All	April 3, 1917	All points outside state
West Virginia...	All	All	April 18, 1917	All points outside state
Wisconsin.....	P. strobus, monticola, lambertiana, cembra, excelsa	None	June 1, 1916	All points outside state

PERLEY SPAULDING AND ROY G. PIERCE

Personals. Arthur S. Rhodes, assistant in forest botany at the New York State College of Forestry, Syracuse, New York, has been appointed assistant in the Office of Forest Pathology, Bureau of Plant Industry.

Miss Ruby J. Tiller, scientific assistant in the Office of Forest Pathology, Bureau of Plant Industry, has resigned her position to become the wife of Prof. S. F. Acree of the University of Wisconsin.

Prof. L. H. Pennington of the New York State College of Forestry, Syracuse, New York, has accepted a temporary position as expert with the Office of Forest Pathology, Bureau of Plant Industry. Doctor Pennington will have charge of the season's work on white pine blister rust eradication in the state of Michigan.

Mr. G. H. Godfrey, of Iowa State College, was appointed scientific assistant in Cotton, Truck and Forage Crop Disease Investigations, Bureau of Plant Industry, effective June 8. Mr. Godfrey was formerly scientific assistant in Cereal Disease Investigations, but during the past year was granted leave of absence to engage in post-graduate study.

LITERATURE ON PLANT DISEASES

COMPILED BY EUNICE R. OBERLY, LIBRARIAN, BUREAU OF PLANT INDUSTRY, AND FLORENCE P. SMITH, ASSISTANT

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